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**UNITED STATES DISTRICT COURT  
DISTRICT OF NEW JERSEY**

**CELGENE CORPORATION,**

**Plaintiff,**

**v.**

**MYLAN PHARMACEUTICALS INC.,  
MYLAN INC., and MYLAN N.V.,**

**Defendants.**

**Civil Action No. \_\_\_\_\_**

**COMPLAINT FOR  
PATENT INFRINGEMENT**

**(Filed Electronically)**

Plaintiff Celgene Corporation (“Celgene”), by its undersigned attorneys, for its Complaint against defendants Mylan Pharmaceuticals Inc., Mylan Inc., and Mylan N.V. (collectively, “Mylan”), alleges as follows:

**Nature of the Action**

1. This is an action for patent infringement under the patent laws of the United States, 35 U.S.C. §100, *et seq.*, arising from Mylan’s filing of Abbreviated New Drug Application (“ANDA”) No. 213912 (“Mylan’s ANDA”) with the United States Food and Drug Administration (“FDA”) seeking approval to commercially market generic versions of Celgene’s REVLIMID<sup>®</sup> drug products prior to the expiration of United States Patent Nos. 7,189,740 (“the ’740 patent”), 7,465,800 (“the ’800 patent”), 7,855,217 (“the ’217 patent”), 7,968,569 (“the ’569

patent”), 8,404,717 (“the ’717 patent”), 8,530,498 (“the ’498 patent”), 8,648,095 (“the ’095 patent”), 9,056,120 (“the ’120 patent”), 9,101,621 (“the ’621 patent”), and 9,101,622 (“the ’622 patent”) (collectively, “the patents-in-suit”), owned by Celgene.

### **The Parties**

2. Plaintiff Celgene is a biopharmaceutical company committed to improving the lives of patients worldwide. Celgene focuses on, and invests heavily in, the discovery and development of products for the treatment of severe and life-threatening conditions. Celgene is a world leader in the treatment of many such diseases, including cancer. Celgene is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at 86 Morris Avenue, Summit, New Jersey 07901.

3. On information and belief, Defendant Mylan N.V. is a corporation organized and existing under the laws of Netherlands, having a place of business at Building 4, Trident Place, Mosquito Way, Hatfield, Hertfordshire, AL109UL, England. On information and belief, the Chief Executive Officer and other executive officers of Mylan N.V. carry out the day-to-day conduct of Mylan N.V.’s worldwide businesses at the company’s principal offices in Canonsburg, Pennsylvania.

4. On information and belief, Defendant Mylan Pharmaceuticals Inc. is a corporation organized and existing under the laws of West Virginia, having a principal place of business at 781 Chestnut Ridge Road, Morgantown, West Virginia 26505.

5. On information and belief, Defendant Mylan Inc. is a corporation organized and existing under the laws of Pennsylvania, having a principal place of business at 1000 Mylan Boulevard, Robert J. Coury Global Center, Canonsburg, Pennsylvania 15317.

6. On information and belief, Mylan Pharmaceuticals Inc. is a wholly owned subsidiary of Mylan Inc.

7. On information and belief, Mylan Inc. is a wholly owned subsidiary of Mylan N.V.

### **The Patents-in-Suit**

8. On March 13, 2007, the United States Patent and Trademark Office (“USPTO”) duly and lawfully issued the ’740 patent, entitled, “Methods of Using 3-(4-amino-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for the Treatment and Management of Myelodysplastic Syndromes,” to Celgene as assignee. A copy of the ’740 patent is attached hereto as Exhibit A.

9. On December 16, 2008, the USPTO duly and lawfully issued the ’800 patent, entitled, “Polymorphic Forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione,” to Celgene as assignee. A copy of the ’800 patent is attached hereto as Exhibit B.

10. On December 21, 2010, the USPTO duly and lawfully issued the ’217 patent, entitled “Polymorphic Forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione,” to Celgene as assignee. A copy of the ’217 patent is attached hereto as Exhibit C.

11. On June 28, 2011, the USPTO duly and lawfully issued the ’569 patent, entitled, “Methods For Treatment of Multiple Myeloma Using 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione,” to Celgene as assignee. A copy of the ’569 patent is attached hereto as Exhibit D.

12. On March 26, 2013, the USPTO duly and lawfully issued the ’717 patent, entitled, “Methods of Treating Myelodysplastic Syndromes Using Lenalidomide,” to Celgene as assignee. A copy of the ’717 patent is attached hereto as Exhibit E.

13. On September 10, 2013, the USPTO duly and lawfully issued the '498 patent, entitled, "Methods For Treating Multiple Myeloma With 3-(4-amino-1-oxo-1,3-dihydroisoindol-2-yl)piperidine-2,6-dione," to Celgene as assignee. A copy of the '498 patent is attached hereto as Exhibit F.

14. On February 11, 2014, the USPTO duly and lawfully issued the '095 patent, entitled, "Methods For Treating Multiple Myeloma Using 3-(4-amino-1-oxo-1,3-dihydroisoindol-2-yl)-piperidine-2,6-dione In Combination With Proteasome Inhibitor," to Celgene as assignee. A copy of the '095 patent is attached hereto as Exhibit G.

15. On June 16, 2015, the USPTO duly and lawfully issued the '120 patent, entitled, "Methods of Treating Myelodysplastic Syndromes with a Combination Therapy Using Lenalidomide and Azacitidine," to Celgene as assignee. A copy of the '120 patent is attached hereto as Exhibit H.

16. On August 11, 2015, the USPTO duly and lawfully issued the '621 patent, entitled, "Methods For Treating Multiple Myeloma With 3-(4-amino-1-oxo-1,3-dihydroisoindol-2-yl)-piperidine-2,6-dione After Stem Cell Transplantation," to Celgene as assignee. A copy of the '621 patent is attached hereto as Exhibit I.

17. On August 11, 2015, the USPTO duly and lawfully issued the '622 patent, entitled, "Methods For Treating Newly Diagnosed Multiple Myeloma 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione In Combination With Dexamethasone," to Celgene as assignee. A copy of the '622 patent is attached hereto as Exhibit J.

**The Revlimid<sup>®</sup> Drug Product**

18. Celgene holds an approved New Drug Application (“NDA”) under Section 505(a) of the Federal Food Drug and Cosmetic Act (“FFDCA”), 21 U.S.C. § 355(a), for lenalidomide capsules (NDA No. 021880), which it sells under the trade name REVLIMID<sup>®</sup>.

19. The claims of the patents-in-suit cover, *inter alia*, solid forms of lenalidomide, pharmaceutical compositions containing lenalidomide, and methods of use and administration of lenalidomide or pharmaceutical compositions containing lenalidomide.

20. Pursuant to 21 U.S.C. § 355(b)(1) and attendant FDA regulations, the patents-in-suit are listed in the FDA publication, “Approved Drug Products with Therapeutic Equivalence Evaluations” (the “Orange Book”), with respect to REVLIMID<sup>®</sup>.

21. The labeling for REVLIMID<sup>®</sup> instructs and encourages physicians, pharmacists, and other healthcare workers and patients to administer REVLIMID<sup>®</sup> according to one or more of the methods claimed in the patents-in-suit.

**Jurisdiction and Venue**

22. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331, 1338(a), 2201, and 2202.

23. This Court has personal jurisdiction over Mylan Pharmaceuticals Inc. by virtue of, *inter alia*, its systematic and continuous contacts with the State of New Jersey. On information and belief, Mylan Pharmaceuticals Inc. is registered with the State of New Jersey’s Division of Revenue and Enterprise Services as a business operating in New Jersey under Business Id. No. 0100214277. On information and belief, Mylan Pharmaceuticals Inc. is registered with the State of New Jersey’s Department of Health as a drug manufacturer and wholesaler under Registration No. 5003762. On information and belief, Mylan Pharmaceuticals Inc. purposefully has

conducted and continues to conduct business in this Judicial District. By virtue of its physical presence in New Jersey, this Court has personal jurisdiction over Mylan Pharmaceuticals Inc.

24. On information and belief, Mylan Pharmaceuticals Inc. is in the business of, among other things, manufacturing, marketing, importing, offering for sale, and selling pharmaceutical products, including generic drug products, throughout the United States, including in this Judicial District. On information and belief, this Judicial District will be a destination for the generic drug products described in Mylan's ANDA. On information and belief, Mylan Pharmaceuticals Inc. also prepares and/or aids in the preparation and submission of ANDAs to the FDA.

25. This Court has personal jurisdiction over Mylan Inc. by virtue of, *inter alia*, its systematic and continuous contacts with the State of New Jersey. On information and belief, Mylan Inc. is registered with the State of New Jersey's Division of Revenue and Enterprise Services as a business operating in New Jersey under Business Id. No. 0100971292. On information and belief, Mylan Inc. purposefully has conducted and continues to conduct business in this Judicial District. By virtue of its physical presence in New Jersey, this Court has personal jurisdiction over Mylan Inc.

26. On information and belief, Mylan Inc. is in the business of, among other things, manufacturing, marketing, importing, offering for sale, and selling pharmaceutical products, including generic drug products, throughout the United States, including in this Judicial District. On information and belief, this Judicial District will be a destination for the generic drug products described in Mylan's ANDA. On information and belief, Mylan Inc. also prepares and/or aids in the preparation and submission of ANDAs to the FDA.

27. This Court has personal jurisdiction over Mylan N.V. because, *inter alia*, it: (1) has purposefully availed itself of the privilege of doing business in New Jersey, including directly or indirectly through its subsidiaries, agents, and/or alter egos, including Mylan Pharmaceuticals Inc. and Mylan Inc., companies registered with the State of New Jersey, and (2) maintains extensive and systematic contacts with the State of New Jersey, including the marketing, distribution, and/or sale of generic pharmaceutical drugs in New Jersey including through, directly or indirectly, Mylan Pharmaceuticals Inc. and Mylan Inc.

28. This Court has personal jurisdiction over Mylan Pharmaceuticals Inc. and Mylan Inc. because, *inter alia*, they: (1) have purposefully availed themselves of the privilege of doing business in New Jersey, including directly or indirectly through their subsidiaries, agents, and/or alter egos, including companies registered with the State of New Jersey; and (2) maintain extensive and systematic contacts with the State of New Jersey, including the marketing, distribution, and/or sale of generic pharmaceutical drugs in New Jersey including through, directly or indirectly, their subsidiaries, agents, and/or alter egos.

29. This Court has personal jurisdiction over Mylan because, *inter alia*, it has committed an act of patent infringement under 35 U.S.C. § 271(e)(2), and has sent notice of that infringement to Celgene in the State of New Jersey. On information and belief, Mylan intends a future course of conduct that includes acts of patent infringement in New Jersey. These acts have led and will continue to lead to foreseeable harm and injury to Celgene in New Jersey and in this Judicial District.

30. Mylan N.V.'s website (<http://www.mylan.com/en/company/corporate-governance>) states that “[t]he Chief Executive Officer and other executive officers of Mylan

N.V. carry out the day-to-day conduct of Mylan N.V.'s worldwide businesses at the company's principal offices in Canonsburg, Pennsylvania.”

31. Mylan N.V.'s Form 10-K Annual Report for the Period Ending 12/13/2016 (“Mylan Annual Report”) states that on February 27, 2015, “Mylan Inc. became an indirect wholly owned subsidiary of Mylan N.V., and Mylan Inc.'s common stock ceased trading on the NASDAQ.” *See* Mylan Annual Report at 53. The Mylan Annual Report further states that “Mylan N.V. is the successor to Mylan Inc.” *Id.* at 55.

32. On information and belief, Mylan Pharmaceuticals Inc., Mylan Inc., and Mylan N.V. work in concert with respect to the regulatory approval, manufacturing, marketing, sale, and distribution of generic pharmaceutical products throughout the United States, including in this Judicial District.

33. On information and belief, Mylan Pharmaceuticals Inc. acts at the direction, and for the benefit, of Mylan N.V. and Mylan Inc., and is controlled and/or dominated by Mylan N.V. and Mylan Inc.

34. On information and belief, members of the Mylan corporate family have locations in or are incorporated in the State of New Jersey. On information and belief, these entities are controlled and/or dominated by Mylan Pharmaceuticals Inc., Mylan Inc., and/or Mylan N.V. and/or are alter egos of Mylan Pharmaceuticals Inc., Mylan Inc., and/or Mylan N.V.

35. On information and belief, Mylan Pharmaceuticals Inc., Mylan Inc., and Mylan N.V., directly or indirectly or through each other or other entities, maintain regular and established places of business in New Jersey.

36. On information and belief, Mylan Inc. and Mylan Pharmaceuticals Inc. have previously been sued in this Judicial District and have not challenged personal jurisdiction. *See*,



*e.g., Baxter Healthcare Corp., et al. v. Agila Specialties Priv. Ltd., et al.*, Civil Action No. 14-7094 (JBS)(JS) (D.N.J.) (Mylan Pharmaceuticals Inc.); *Astrazeneca AB, et al. v. Mylan Pharm., Inc., et al.*, Civil Action No. 13-4022 (MLC)(DEA) (D.N.J.) (Mylan Pharmaceuticals Inc. and Mylan Inc.); *Janssen Prods., L.P., et al. v. Lupin Ltd., et al.*, Civil Action No. 10-5954 (WHW)(CLW) (D.N.J.) (Mylan Pharmaceuticals Inc. and Mylan Inc.).

37. Mylan Inc. and Mylan Pharmaceuticals Inc. have further availed themselves of the jurisdiction of this Court by previously initiating litigation in this Judicial District. *See, e.g., Mylan Pharm., Inc. v. Celgene Corp.*, Civil Action No. 14-2094 (ES)(MAH) (D.N.J.); *Mylan Inc., et al. v. Apotex Inc., et al.*, Civil Action No. 14-4560 (MAS)(LHG) (D.N.J.).

38. Venue is proper in this Judicial District pursuant to 28 U.S.C. §§ 1391 and/or 1400(b).

#### **Acts Giving Rise To This Suit**

39. Pursuant to Section 505 of the FFDCA, Mylan filed Mylan's ANDA seeking approval to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of lenalidomide capsules 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg, and 25 mg ("Mylan's Proposed Products"), before the patents-in-suit expire.

40. On information and belief, following FDA approval of Mylan's ANDA, Mylan will make, use, offer for sale, or sell Mylan's Proposed Products throughout the United States, or import such generic products into the United States.

41. On information and belief, in connection with the filing of its ANDA as described above, Mylan provided a written certification to the FDA, pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV) ("Mylan's Paragraph IV Certification"), alleging that the claims of the '740 patent, the '800 patent, the '217 patent, the '569 patent, the '717 patent, the '498 patent, the

'095 patent, the '120 patent, and the '622 patent are invalid, unenforceable, and/or will not be infringed by the activities described in Mylan's ANDA.

42. No earlier than November 20, 2019, Mylan sent written notice of its Paragraph IV Certification to Celgene ("Mylan's Notice Letter"). Mylan's Notice Letter alleged that the claims of the '740 patent, the '800 patent, the '217 patent, the '569 patent, the '717 patent, the '498 patent, the '095 patent, the '120 patent, and the '622 patent are invalid, unenforceable, and/or will not be infringed by the activities described in Mylan's ANDA. Mylan's Notice Letter also informed Celgene that Mylan seeks approval to market Mylan's Proposed Products before the patents-in-suit expire. Mylan specifically directed Mylan's Notice Letter to Celgene's headquarters in Summit, New Jersey, in this Judicial District.

**Count I: Infringement of the '740 Patent**

43. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

44. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '740 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

45. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '740 patent.

46. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '740 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

47. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '740 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will intentionally encourage acts of direct infringement with knowledge of the '740 patent and knowledge that its acts are encouraging infringement.

48. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '740 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of the '740 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

49. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '740 patent is not enjoined.

50. Celgene does not have an adequate remedy at law.

51. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

### **Count II: Infringement of the '800 Patent**

52. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

53. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to

the expiration of the '800 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

54. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '800 patent.

55. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '800 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

56. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '800 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will intentionally encourage acts of direct infringement with knowledge of the '800 patent and knowledge that its acts are encouraging infringement.

57. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '800 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of the '800 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

58. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '800 patent is not enjoined.

59. Celgene does not have an adequate remedy at law.

60. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

**Count III: Infringement of the '217 Patent**

61. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

62. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '217 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

63. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '217 patent.

64. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '217 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

65. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '217 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will intentionally encourage acts of direct infringement with knowledge of the '217 patent and knowledge that its acts are encouraging infringement.

66. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '217 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the

United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of the '217 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

67. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '217 patent is not enjoined.

68. Celgene does not have an adequate remedy at law.

69. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

#### **Count IV: Infringement of the '569 Patent**

70. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

71. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '569 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

72. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '569 patent.

73. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '569 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

74. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '569 patent under 35 U.S.C. § 271(b) by

making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will intentionally encourage acts of direct infringement with knowledge of the '569 patent and knowledge that its acts are encouraging infringement.

75. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '569 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of the '569 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

76. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '569 patent is not enjoined.

77. Celgene does not have an adequate remedy at law.

78. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

**Count V: Infringement of the '717 Patent**

79. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

80. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '717 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

81. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '717 patent.

82. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '717 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

83. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '717 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will intentionally encourage acts of direct infringement with knowledge of the '717 patent and knowledge that its acts are encouraging infringement.

84. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '717 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of the '717 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

85. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '717 patent is not enjoined.

86. Celgene does not have an adequate remedy at law.

87. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.



**Count VI: Infringement of the '498 Patent**

88. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

89. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '498 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

90. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '498 patent.

91. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '498 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

92. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '498 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will intentionally encourage acts of direct infringement with knowledge of the '498 patent and knowledge that its acts are encouraging infringement.

93. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '498 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of

the '498 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

94. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '498 patent is not enjoined.

95. Celgene does not have an adequate remedy at law.

96. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

**Count VII: Infringement of the '095 Patent**

97. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

98. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '095 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

99. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '095 patent.

100. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '095 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

101. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '095 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will

intentionally encourage acts of direct infringement with knowledge of the '095 patent and knowledge that its acts are encouraging infringement.

102. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '095 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of the '095 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

103. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '095 patent is not enjoined.

104. Celgene does not have an adequate remedy at law.

105. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

#### **Count VIII: Infringement of the '120 Patent**

106. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

107. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '120 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

108. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '120 patent.

109. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '120 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

110. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '120 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will intentionally encourage acts of direct infringement with knowledge of the '120 patent and knowledge that its acts are encouraging infringement.

111. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '120 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of the '120 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

112. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '120 patent is not enjoined.

113. Celgene does not have an adequate remedy at law.

114. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

**Count IX: Infringement of the '621 Patent**

115. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

116. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '621 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

117. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '621 patent.

118. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '621 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

119. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '621 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will intentionally encourage acts of direct infringement with knowledge of the '621 patent and knowledge that its acts are encouraging infringement.

120. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '621 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of

the '621 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

121. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '621 patent is not enjoined.

122. Celgene does not have an adequate remedy at law.

123. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

**Count X: Infringement of the '622 Patent**

124. Celgene repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

125. Mylan's submission of its ANDA to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Mylan's Proposed Products, prior to the expiration of the '622 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

126. There is a justiciable controversy between Celgene and Mylan as to the infringement of the '622 patent.

127. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will infringe one or more claims of the '622 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States.

128. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will induce infringement of one or more claims of the '622 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, upon FDA approval of Mylan's ANDA, Mylan will

intentionally encourage acts of direct infringement with knowledge of the '622 patent and knowledge that its acts are encouraging infringement.

129. Unless enjoined by this Court, upon FDA approval of Mylan's ANDA, Mylan will contributorily infringe one or more claims of the '622 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Mylan's Proposed Products in the United States. On information and belief, Mylan has had and continues to have knowledge that Mylan's Proposed Products are especially adapted for a use that infringes one or more claims of the '622 patent and that there is no substantial non-infringing use for Mylan's Proposed Products.

130. Celgene will be substantially and irreparably damaged and harmed if Mylan's infringement of the '622 patent is not enjoined.

131. Celgene does not have an adequate remedy at law.

132. This case is an exceptional one, and Celgene is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

### **PRAYER FOR RELIEF**

WHEREFORE, Plaintiff Celgene respectfully requests the following relief:

(A) A Judgment that Mylan has infringed the patents-in-suit by submitting ANDA No. 213912;

(B) A Judgment that Mylan has infringed, and that Mylan's making, using, offering to sell, selling, or importing Mylan's Proposed Products will infringe one or more claims of the patents-in-suit;

(C) An Order that the effective date of FDA approval of ANDA No. 213912 be a date which is not earlier than the later of the expiration of the patents-in-suit, or any later expiration of exclusivity to which Celgene is or becomes entitled;

(D) Preliminary and permanent injunctions enjoining Mylan and its officers, agents, attorneys and employees, and those acting in privity or concert with them, from making, using, offering to sell, selling, or importing Mylan's Proposed Products until after the expiration of the patents-in-suit, or any later expiration of exclusivity to which Celgene is or becomes entitled;

(E) A permanent injunction, pursuant to 35 U.S.C. § 271(e)(4)(B), restraining and enjoining Mylan, its officers, agents, attorneys and employees, and those acting in privity or concert with them, from practicing any solid forms of lenalidomide, compositions, or methods claimed in the patents-in-suit, or from actively inducing or contributing to the infringement of any claim of the patents-in-suit, until after the expiration of the patents-in-suit, or any later expiration of exclusivity to which Celgene is or becomes entitled;

(F) A Judgment that the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of Mylan's Proposed Products will directly infringe, induce and/or contribute to infringement of the patents-in-suit;

(G) To the extent that Mylan has committed any acts with respect to the solid forms of lenalidomide, compositions, methods claimed in the patents-in-suit, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), a Judgment awarding Celgene damages for such acts;

(H) If Mylan engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of Mylan's Proposed Products prior to the expiration of the



patents-in-suit, a Judgment awarding damages to Celgene resulting from such infringement, together with interest;

(I) A Judgment declaring that the patents-in-suit remain valid and enforceable;

(J) A Judgment that this is an exceptional case pursuant to 35 U.S.C. § 285 and awarding Celgene its attorneys' fees incurred in this action;

(K) A Judgment awarding Celgene its costs and expenses incurred in this action; and

(L) Such further and other relief as this Court may deem just and proper.

Dated: December 31, 2019

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**CERTIFICATION PURSUANT TO L. CIV. R. 11.2 & 40.1**

I further certify that the matters captioned *Celgene Corporation v. Dr. Reddy's Laboratories, Ltd., et al.*, Civil Action No. 16-7704 (SDW)(LDW) (D.N.J.), *Celgene Corporation v. Dr. Reddy's Laboratories, Ltd., et al.*, Civil Action No. 17-5314 (SDW)(LDW) (D.N.J.), *Celgene Corporation v. Zydus Pharmaceuticals (USA) Inc., et al.*, Civil Action No. 17-2528 (SDW)(LDW) (D.N.J.), *Celgene Corporation v. Cipla Limited*, Civil Action No. 17-6163 (SDW)(LDW) (D.N.J.), *Celgene Corporation v. Cipla Limited*, Civil Action No. 19-14731 (SDW)(LDW) (D.N.J.), *Celgene Corporation v. Apotex Inc.*, Civil Action No. 18-461 (SDW)(LDW) (D.N.J.), *Celgene Corporation v. Apotex Inc.*, Civil Action No. 19-6999 (SDW)(LDW) (D.N.J.), *Celgene Corporation v. Sun Pharmaceutical Industries, Inc., et al.*, Civil Action No. 18-11630 (SDW)(LDW) (D.N.J.), *Celgene Corporation v. Hetero Labs Limited, et al.*, Civil Action No. 18-17463 (SDW)(LDW) (D.N.J.), and *Celgene Corporation v. Hetero Labs Limited, et al.*, Civil Action No. 19-15449 (SDW)(LDW) (D.N.J.) are related to the matter in controversy because the matter in controversy involves the same plaintiff and some of the same patents, and because the defendants are seeking FDA approval to market generic versions of the same pharmaceutical product.

I further certify that, to the best of my knowledge, the matter in controversy is not the subject of any other action pending in any court, or of any pending arbitration or administrative proceeding.

Dated: December 31, 2019

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# **EXHIBIT A**

US007189740B2

(12) **United States Patent**  
**Zeldis**(10) **Patent No.:** **US 7,189,740 B2**  
(45) **Date of Patent:** **\*Mar. 13, 2007**(54) **METHODS OF USING  
3-(4-AMINO-OXO-1,3-DIHYDRO-ISOINDOL-  
2-YL)-PIPERIDINE-2,6-DIONE FOR THE  
TREATMENT AND MANAGEMENT OF  
MYELOYDYSPLASTIC SYNDROMES**(75) Inventor: **Jerome B. Zeldis**, Princeton, NJ (US)(73) Assignee: **Celgene Corporation**, Summit, NJ  
(US)(\*) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.This patent is subject to a terminal dis-  
claimer.(21) Appl. No.: **10/411,649**(22) Filed: **Apr. 11, 2003**(65) **Prior Publication Data**

US 2004/0220144 A1 Nov. 4, 2004

**Related U.S. Application Data**(60) Provisional application No. 60/418,468, filed on Oct.  
15, 2002.(51) **Int. Cl.****A61K 31/47** (2006.01)**A61K 31/445** (2006.01)(52) **U.S. Cl.** ..... **514/323; 514/319; 514/58**(58) **Field of Classification Search** ..... **514/323,**  
**514/58, 319**

See application file for complete search history.

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*Primary Examiner*—Vickie Kim(74) *Attorney, Agent, or Firm*—Jones Day(57) **ABSTRACT**

Methods of treating, preventing and/or managing myelodysplastic syndromes are disclosed. Specific methods encompass the administration of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, alone or in combination with a second active ingredient, and/or the transplantation of blood or cells. Specific second active ingredients are capable of affecting or blood cell production. Pharmaceutical compositions, single unit dosage forms, and kits suitable for use in methods of the invention are also disclosed.

**34 Claims, No Drawings**

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**METHODS OF USING  
3-(4-AMINO-OXO-1,3-DIHYDRO-ISOINDOL-  
2-YL)-PIPERIDINE-2,6-DIONE FOR THE  
TREATMENT AND MANAGEMENT OF  
MYELOYDYSPLASTIC SYNDROMES**

This application claims priority to U.S. Provisional Patent Application No. 60/418,468 filed on Oct. 15, 2002, the entirety of which is incorporated herein by reference.

## 1. FIELD OF THE INVENTION

This invention relates to methods of treating, preventing and/or managing myelodysplastic and related syndromes which comprise the administration of immunomodulatory compounds alone or in combination with known therapeutics. The invention also relates to pharmaceutical compositions and dosing regimens. In particular, the invention encompasses the use of immunomodulatory compounds in conjunction with transplantation therapy and/or other standard therapies for myelodysplastic syndromes.

## 2. BACKGROUND OF THE INVENTION

### 2.1. Pathobiology of MDS

Myelodysplastic syndrome ("MDS") refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. *The Merck Manual* 953 (17<sup>th</sup> ed. 1999) and List et al., 1990, *J Clin. Oncol.* 8:1424.

The initial hematopoietic stem cell injury can be from causes such as, but not limited to, cytotoxic chemotherapy, radiation, virus, chemical exposure, and genetic predisposition. A clonal mutation predominates over bone marrow, suppressing healthy stem cells. In the early stages of MDS, the main cause of cytopenias is increased programmed cell death (apoptosis). As the disease progresses and converts into leukemia, gene mutation rarely occurs and a proliferation of leukemic cells overwhelms the healthy marrow. The disease course differs, with some cases behaving as an indolent disease and others behaving aggressively with a very short clinical course that converts into an acute form of leukemia.

The actual incidence of MDS in the U.S. is unknown. MDS was first considered a distinct disease in 1976, and occurrence was estimated at 1500 new cases every year. At that time, only patients with less than five percent blasts were considered to have this disorder. Statistics from 1999 estimated 13,000 new cases per year and about 1000 cases per year in children, surpassing chronic lymphocytic leukemia as the most common form of leukemia in the western hemisphere. The perception that the incidence is increasing may be due to improvements in recognition and criteria for diagnosis. The disease is found worldwide.

An international group of hematologists, the French-American-British (FAB) Cooperative Group, classified MDS disorders into five subgroups, differentiating them from acute myeloid leukemia. *The Merck Manual* 954 (17<sup>th</sup> ed. 1999); Bennett J. M., et al., *Ann. Intern. Med.* 1985 October, 103(4): 620-5; and Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599-617. An underlying trilineage dysplastic change in the bone marrow cells of the patients is found in all subtypes.

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There are two subgroups of refractory anemia characterized by five percent or less myeloblasts in bone marrow: (1) refractory anemia (RA) and; (2) RA with ringed sideroblasts (RARS), defined morphologically as having 15% erythroid cells with abnormal ringed sideroblasts, reflecting an abnormal iron accumulation in the mitochondria. Both have a prolonged clinical course and low incidence of progression to acute leukemia. Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599-617.

There are two subgroups of refractory anemias with greater than five percent myeloblasts: (1) RA with excess blasts (RAEB), defined as 6-20% myeloblasts, and (2) RAEB in transformation (RAEB-T), with 21-30% myeloblasts. The higher the percentage of myeloblasts, the shorter the clinical course and the closer the disease is to acute myelogenous leukemia. Patient transition from early to more advanced stages indicates that these subtypes are merely stages of disease rather than distinct entities. Elderly patients with MDS with trilineage dysplasia and greater than 30% myeloblasts who progress to acute leukemia are often considered to have a poor prognosis because their response rate to chemotherapy is lower than de novo acute myeloid leukemia patients. The World Health Organization (WHO) classification (1999) proposes to include all cases of RAEB-T, or patients with greater than 20% myeloblasts, in the category of acute leukemia because these patients have similar prognostic outcomes. However, their response to therapy is worse than the de novo or more typical acute myelogenous leukemia or acute nonlymphocytic leukemia (ANLL) patient. Id.

The fifth type of MDS, the most difficult to classify, is called chronic myelomonocytic leukemia (CMML). This subtype can have any percentage of myeloblasts but presents with a monocytosis of 1000/dL or more. It may be associated with splenomegaly. This subtype overlaps with a myeloproliferative disorder and may have an intermediate clinical course. It is differentiated from the classic chronic myelocytic leukemia (CML) that is characterized by a negative Ph chromosome. The recent WHO classification (1999) proposes that juvenile and proliferative CMML be listed separately from FAB under MDS/myeloproliferative disorder (MPD) with splenomegaly and greater than 13,000 total WBC. CMML is limited to monocytosis, less than 13,000/mm<sup>3</sup> total leukocytes, and requires trilineage dysplasia. Id. Harris N. L., et al., *J. Clin. Oncol.* 1999 December, 17(12): 3835-49. Finally, some other international organizations, including WHO, have suggested a sixth class of MDS patients, characterized by a del (5q) abnormality.

MDS is primarily a disease of elderly people, with the median onset in the seventh decade of life. The median age of these patients is 65 years, with ages ranging from the early third decade of life to as old as 80 years or older. The syndrome may occur in any age group, including the pediatric population. Patients who survive malignancy treatment with alkylating agents, with or without radiotherapy, have a high incidence of developing MDS or secondary acute leukemia. About 60-70% of patients do not have an obvious exposure or cause for MDS, and are classified as primary MDS patients.

The most common cases of MDS are primary, or idiopathic. However, a nonspecific history of exposure to indeterminable chemicals or radiation 10-15 years prior to onset of disease may be present in about 50% of patients. This relationship to pathogenesis remains unproved. Compounds such as, but not limited to, benzene, insecticides, weed killers, and fungicides are possible causes of MDS. Goldberg H., et al., *Cancer Res.* 1990 Nov. 1; 50(21): 6876-81.

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Secondary MDS describes development of MDS or acute leukemia after known exposures to chemotherapy drugs that can cause bone marrow damage. These drugs are associated with a high incidence of chromosomal abnormalities following exposure and at the time of MDS or acute leukemia diagnosis.

Further, MDS is associated with complications associated with severe cytopenias. Other complications are development of myelofibrosis, which can accelerate decline in blood counts and increase transfusion requirements. Transformation to acute leukemia accelerates the development of complications such as anemia, bleeding, and infections.

Recently, the International MDS Risk Analysis (IMRA) Workshop proposed an International Prognosis Scoring System (IPSS) to decrease imprecision in predicting survival and AML risk in MDS patients. The IPSS is based on the number of cytopenias, percentage of BM blasts, and type of cytogenetic abnormalities (Table 1). Greenberg et al., *Blood* 1997, 89:2079–88. The latter are categorized into good (normal, -Y, del (5q), del (20q)), intermediate, and poor subgroups (complex or chromosome 7 abnormalities).

TABLE 1

International Prognostic Scoring System for MDS					
	Score Value				
Prognostic Variable	0	0.5	1.0	1.5	2.0
Bonemarrow blasts (%)	<5	5–10	—	11–20	21–30
Karyotype*	Good	Intermediate	Poor		
Cytopenias	0/1	2/3			

\*Good, normal, del (5q), del (20q), -Y; Poor, complex (>3) or chromosome 7 abnormalities; Intermediate, +8, and other single or double abnormalities.

## 2.2. MDS Treatment

The current treatment of MDS is based on the stage and the mechanism of the disease that predominates the particular phase of the disease process. Bone marrow transplantation has been used in patients with poor prognosis or late-stage MDS. Epstein and Slease, 1985, *Surg. Ann.* 17:125. This type of therapy, however, is both painful for donor and recipient, because of the involvement of invasive procedures and can cause severe and even fatal complications to the recipient, particularly with allogeneic transplant and related Graft Versus Host Disease (GVHD) results. Therefore, the risk of GVHD restricts the use of bone marrow transplantation to patients with otherwise fatal diseases. Further, as most patients are elderly and only a few young MDS patients will have a matched donor, the use of bone marrow transplantation is limited.

An alternative approach to therapy for MDS is the use of hematopoietic growth factors or cytokines to stimulate blood cell development in a recipient. Dexter, 1987, *J. Cell Sci.* 88:1; Moore, 1991, *Annu. Rev. Immunol.* 9:159; and Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599–617. The process of blood cell formation, by which a small number of self-renewing stem cells give rise to lineage specific progenitor cells that subsequently undergo proliferation and differentiation to produce the mature circulating blood cells has been shown to be at least in part regulated by specific hormones. These hormones are collectively known as hematopoietic growth factors. Metcalf, 1985, *Science* 229: 16; Dexter, 1987, *J. Cell Sci.* 88:1; Golde and Gasson, 1988, *Scientific American*, July:62; Tabbara and Robinson, 1991, *Anti-Cancer Res.* 11:81; Ogawa, 1989, *Environ. Health*

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*Presp.* 80:199; and Dexter, 1989, *Br. Med. Bull.* 45:337. The most well characterized growth factors include erythropoietin (EPO), granulocyte macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF). Apart from inducing proliferation and differentiation of hematopoietic progenitor cells, such cytokines have also been shown to activate a number of functions of mature blood cells, including influencing the migration of mature hematopoietic cells. Stanley et al., 1976, *J. Exp. Med.* 143:631; Schrader et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:323; Moore et al., 1980, *J. Immunol.* 125:1302; Kurland et al., 1979, *Proc. Natl. Acad. Sci. U.S.A.* 76:2326; Handman and Burgess, 1979, *J. Immunol.* 122:1134; Vadas et al., 1983, *Blood* 61:1232; Vadas et al., 1983, *J. Immunol.* 130:795; and Weibart et al., 1986, *J. Immunol.* 137:3584.

Unfortunately, hematopoietic growth factors have not proven effective in many clinical settings. Clinical trials of MDS patients treated with recombinant human GM-CSF and G-CSF have shown that while these cytokines can restore granulocytopenia in treated patients, their efficacy is restricted to the granulocyte or monocyte lineage with little or no improvement in hemoglobin or platelet counts. Schuster et al., 1990, *Blood* 76 (Suppl.1):318a. When such patients were treated with recombinant human EPO, a sustained improvement in hemoglobin or decrease in transfusion requirement was achieved in only less than 25% of patients. Besa et al., 1990, 76 (Suppl.1):133a; Hellstrom et al., 1990, 76 (Suppl.1):279a; Bowen et al., 1991, *Br. J. Haematol.* 77:419. Therefore, there remains a need for safe and effective methods of treating and managing MDS.

## 2.3. Thalidomide and Other Compounds Useful in the Treatment of Disease

Thalidomide is a racemic compound sold under the trade-name Thalomid® and chemically named  $\alpha$ -(N-phthalimido) glutarimide or 2-(2,6-dioxo-3-piperidinyl)-1H-isindole-1,3 (2H)-dione. Thalidomide was originally developed in the 1950's to treat morning sickness, but due to its teratogenic effects was withdrawn from use. Thalidomide has been approved in the United States for the acute treatment of the cutaneous manifestations of erythema nodosum leprosum in leprosy. Physicians' Desk Reference, 1154–1158 (56th ed., 2002). Because its administration to pregnant women can cause birth defects, the sale of thalidomide is strictly controlled. Id. Thalidomide has reportedly been studied in the treatment of other diseases, such as chronic graft-vs-host disease, rheumatoid arthritis, sarcoidosis, several inflammatory skin diseases, and inflammatory bowel disease. See generally, Koch, H. P., *Prog. Med. Chem.* 22:165–242 (1985). See also, Moller, D. R., et al., *J. Immunol.* 159: 5157–5161 (1997); Vasiliauskas, E. A., et al., *Gastroenterology* 117:1278–1287 (1999); Ehrenpreis, E. D., et al., *Gastroenterology* 117:1271–1277 (1999). It has further been alleged that thalidomide can be combined with other drugs to treat ischemia/reperfusion associated with coronary and cerebral occlusion. See U.S. Pat. No. 5,643,915, which is incorporated herein by reference.

More recently, thalidomide was found to exert immunomodulatory and anti-inflammatory effects in a variety of disease states, cachexia in AIDS, and opportunistic infections in AIDS. In studies to define the physiological targets of thalidomide, the drug was found to have a wide variety of biological activities exclusive of its sedative effect including neurotoxicity, teratogenicity, suppression of TNF- $\alpha$  production by monocytes/macrophages and the accompanying inflammatory toxicities associated with high levels of TNF- $\alpha$ , and inhibition of angiogenesis and neovascularization.

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Additionally, beneficial effects have been observed in a variety of dermatological conditions, ulcerative colitis, Crohn's disease, Bechets's syndrome, systemic lupus erythematosus, aphthous ulcers, and lupus. The anti-angiogenic properties of thalidomide in in vivo models have been reported. D'Amato et al., *Thalidomide Is An Inhibitor Of Angiogenesis*, 1994, *PNAS, USA* 91:4082-4085.

One of the most therapeutically significant potential uses of thalidomide is in the treatment of cancer. The compound has been investigated in the treatment of various types of cancer, such as refractory multiple myeloma, brain, breast, colon, and prostate cancer, melanoma, mesothelioma, and renal cell carcinoma. See, e.g., Singhal, S., et al., *New England J. Med.* 341(21):1565-1571 (1999); and Marx, G. M., et al., *Proc. Am. Soc. Clin. Oncology* 18:454a (1999). Thalidomide reportedly can also be used to prevent the development of chronic cardiomyopathy in rats caused by doxorubicin. Costa, P. T., et al., *Blood* 92(10:suppl. 1):235b (1998). Other reports concerning the use of thalidomide in the treatment of specific cancers include its combination with carboplatin in the treatment of glioblastoma multiforme. McCann, J., *Drug Topics* 41-42 (Jun. 21, 1999). The use of thalidomide in combination with dexamethasone reportedly was effective in the treatment of patients suffering from multiple myeloma who also received, as supportive care, human granulocyte colony-stimulating factor (G-CSF), ciprofloxacin, and non-absorbable antifungal agents. Kropff, M. H., *Blood* 96(11 part 1):168a (2000); see also, Munshi, N. et al., *Blood* 94(10 part 1):578a (1999). Other chemotherapy combinations that comprise thalidomide are disclosed in International Application No. PCT/US01/15326 to R. Govindarajan and A. Zeitlan, and in International Application No. PCT/US01/15327 to J. B. Zeldis, et al.

In an effort to provide compounds that have greater therapeutic safety and efficacy than thalidomide, researchers have begun investigating a large number of other compounds, some of which are derivatives of thalidomide. See, e.g., Marriott, J. B., et al., *Expert Opin. Biol. Ther.* 1(4):1-8 (2001); G. W. Muller, et al., *Journal of Medicinal Chemistry* 39(17): 3238-3240 (1996); and G. W. Muller, et al., *Bioorganic & Medicinal Chemistry Letters* 8: 2669-2674 (1998). Examples include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles described in U.S. Pat. Nos. 6,281,230 and 6,316,471, both to G. W. Muller, et al.

A group of compounds selected for their capacity to potentially inhibit TNF- $\alpha$  production by LPS stimulated PBMC has been investigated. L. G. Corral, et al., *Ann. Rheum. Dis.* 58:(Suppl 1) 1107-1113 (1999). These compounds, which are referred to as IMiDs™ or Immunomodulatory Drugs, show not only potent inhibition of TNF- $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  and IL12 production. LPS induced IL6 is also inhibited by IMiDs™, albeit partially. These compounds are potent stimulators of LPS induced IL10, increasing IL10 levels by 200 to 300%. Id.

While many such compounds have shown promise as therapeutic agents, their mechanisms of action and effectiveness are still under investigation. Moreover, there remains a need for therapeutic agents to treat MDS and its related disorders.

### 3. SUMMARY OF THE INVENTION

This invention encompasses methods of treating or preventing myelodysplastic syndrome ("MDS") which com-

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prise administering to a patient in need thereof a therapeutically or prophylactically effective amount of an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. The invention also encompasses methods of managing MDS (e.g., lengthening the time of remission) which comprise administering to a patient in need of such management a therapeutically or prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

One embodiment of the invention encompasses the use of one or more immunomodulatory compounds in combination with conventional therapies presently used to treat, prevent or manage MDS such as hematopoietic growth factors, cytokines, cancer chemotherapeutics, stem cell transplantation and other transplantations.

The invention further encompasses pharmaceutical compositions, single unit dosage forms, and kits suitable for use in treating, preventing and/or managing MDS, which comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

### 4. DETAILED DESCRIPTION OF THE INVENTION

A first embodiment of the invention encompasses methods of treating or preventing MDS which comprise administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. The embodiment encompasses the treatment, prevention or management of specific sub-types of MDS such as refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia.

As used herein, the term "myelodysplastic syndromes" or "MDS" means hematopoietic stem cell disorders characterized by one or more of the following: ineffective blood cell production, progressive cytopenias, risk of progression to acute leukemia or cellular marrow with impaired morphology and maturation (dysmyelopoiesis). The term "myelodysplastic syndromes" or "MDS" unless otherwise noted includes: refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia.

Another embodiment of the invention encompasses methods of managing MDS which comprises administering to a patient in need of such management a prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

Another embodiment of the invention encompasses a pharmaceutical composition comprising an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

Also encompassed by the invention are single unit dosage forms comprising an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

Another embodiment of the invention encompasses a kit comprising: a pharmaceutical composition comprising an



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immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof and a second active or dexamethasone or instructions for use. The invention further encompasses kits comprising single unit dosage forms.

One embodiment of the invention encompasses a method of treating, preventing and/or managing MDS, which comprises administering to a patient in need of such treatment, prevention and/or management a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a therapeutically or prophylactically effective amount of a second active agent.

The second active agent is preferably a hematopoietic growth factor, a cytokine, an anti-cancer agent, an antibiotic, an anti-fungal, an anti-inflammatory, an immunosuppressive agent such as a cyclosporin, conventional therapy for MDS, or other chemotherapeutic agent found for example in the Physician's Desk Reference 2002. Preferred anti-cancer or cancer chemotherapeutics are apoptosis inducing agents, topoisomerase inhibitors, anti-angiogenesis compounds, microtubule stabilizing agents, alkylating agents and other known conventional cancer chemotherapy. Most preferred second active agents are those capable of affecting or improving blood production. Second active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules). The examples of specific second active agent include, but are not limited to, etanercept (Enbrel®), imatinib (Gleevec®), anti-TNF- $\alpha$  antibodies, infliximab (Remicade®), G-CSF, GM-CSF, EPO, topotecan, irinotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, vinblastine, isotretinoin, and 13-cis-retinoic acid. This invention also encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term "mutants" are proteins that lack carbohydrate moieties normally present in their naturally occurring forms (e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001). Vaccines that cause the secretion of proteins disclosed herein as well as pharmacologically active mutants, derivatives, and fusion thereof are also encompassed by the invention.

Without being limited by theory, it is believed that certain immunomodulatory compounds and proteins can act in complementary or synergistic ways in the treatment or management of MDS. It is also believed that certain proteins may reduce or eliminate particular adverse effects associated with some immunomodulatory compounds, thereby allowing the administration of larger amounts of an immunomodulatory compound to patients and/or increasing patient compliance. It is further believed that some immunomodulatory compounds may reduce or eliminate particular adverse effects associated with some protein-based MDS

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therapies, thereby allowing the administration of larger amounts of protein to patients and/or increasing patient compliance.

Another embodiment of the invention encompasses a method of reversing, reducing or avoiding an adverse effect associated with the administration of a chemotherapeutics or therapeutics used to treat cancer or MDS in a patient suffering from MDS, which comprises administering to a patient in need thereof a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

As inevitable leukemic transformation develops in certain stages of MDS, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. It is believed that the combined use of an immunomodulatory compound and transplantation of stem cells in a patient suffering from MDS provides a unique and unexpected synergism. In particular, without being limited by theory, it is believed that an immunomodulatory compound exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy. Immunomodulatory compounds can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of related Graft Versus Host Disease (GVHD). Therefore, this invention encompasses a method of treating, preventing and/or managing MDS, which comprises administering to a patient (e.g., a human) an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after transplantation therapy.

The invention also encompasses pharmaceutical compositions, single unit dosage forms, and kits which comprise one or more immunomodulatory compounds, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, a second active ingredient, and/or blood or cells for transplantation therapy. For example, the kit may contain one or more compounds of the invention, stem cells for transplantation and an immunosuppressive agent, antibiotic or other drug, each of which is to be used to treat the MDS patient.

#### 4.1. Immunomodulatory Compounds

Compounds used in the invention include immunomodulatory compounds that are racemic, stereomerically enriched or stereomerically pure, and pharmaceutically acceptable salts, solvates, hydrates, stereoisomers, clathrates, and prodrugs thereof. Preferred compounds used in the invention are small organic molecules having a molecular weight less than about 1000 g/mol, and are not proteins, peptides, oligonucleotides, oligosaccharides or other macromolecules.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the

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compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. As used herein and unless otherwise indicated, the term "stereomerically enriched" means a composition that comprises greater than about 60% by weight of one stereoisomer of a compound, preferably greater than about 70% by weight, more preferably greater than about 80% by weight of one stereoisomer of a compound. As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center. Similarly, the term "stereomerically enriched" means a stereomerically enriched composition of a compound having one chiral center.

As used herein and unless otherwise indicated, the term "immunomodulatory compounds" or "IMiDs™" (Celgene Corporation) used herein encompasses small organic molecules that markedly inhibit TNF- $\alpha$ , LPS induced monocyte IL1 $\beta$  and IL12, and partially inhibit IL6 production. Specific immunomodulatory compounds of the invention are discussed below.

TNF- $\alpha$  is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation. TNF- $\alpha$  is responsible for a diverse range of signaling events within cells. TNF- $\alpha$  may play a pathological role in cancer. Without being limited by particular theory, one of the biological effects exerted by the immunomodulatory compounds of the invention is the reduction of synthesis of TNF- $\alpha$ . Immunomodulatory compounds of the invention enhance the degradation of TNF- $\alpha$  mRNA.

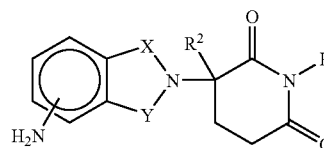
Further, without being limited by particular theory, immunomodulatory compounds used in the invention may also be potent co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. Immunomodulatory compounds of the invention may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset. In addition, the compounds preferably have anti-inflammatory properties, and efficiently co-stimulate T cells.

Specific examples of immunomodulatory compounds of the invention, include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl) isoindolines such as those described in U.S. Pat. No. 5,874,448; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolines described in U.S. Pat. No. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines (e.g., 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517; and a class of non-polypeptide cyclic amides disclosed in U.S. Pat. Nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S. Pat. Nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles such as those described in U.S. Pat. Nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those

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described in U.S. patent application Ser. No. 09/972,487 filed on Oct. 5, 2001, U.S. patent application Ser. No. 10/032,286 filed on Dec. 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents identified herein are incorporated herein by reference. Immunomodulatory compounds of the invention do not include thalidomide.

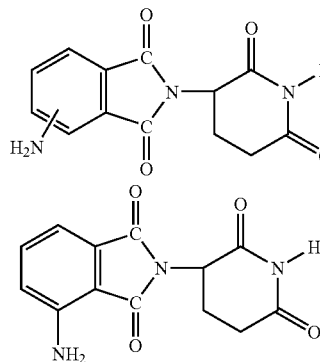
Other specific immunomodulatory compounds of the invention include, but are not limited to, 1-oxo-and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein. These compounds have the structure 1:



in which one of X and Y is C=O, the other of X and Y is C=O or CH<sub>2</sub>, and R<sup>2</sup> is hydrogen or lower alkyl, in particular methyl. Specific immunomodulatory compounds include, but are not limited to:

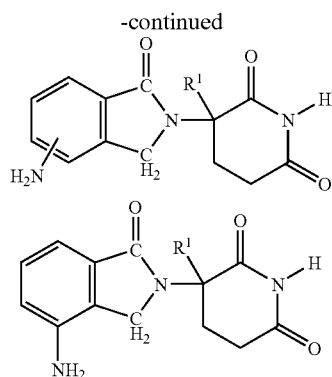
1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;  
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

Other specific immunomodulatory compounds of the invention belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles, such as those described in U.S. Pat. Nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (International Publication No. WO 98/03502), each of which is incorporated herein. Compounds representative of this class are of the formulas:



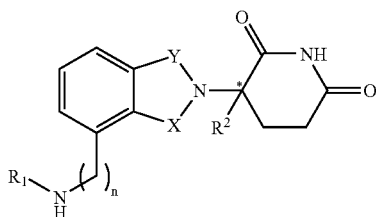
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wherein  $R^1$  is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (e.g. optically pure (R) or (S) enantiomers) of these compounds.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. Nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are incorporated herein by reference. Representative compounds are of formula II:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is  $C=O$  and the other is  $CH_2$  or  $C=O$ ;  $R^1$  is H,  $(C_1-C_8)$  alkyl,  $(C_3-C_7)$  cycloalkyl,  $(C_2-C_8)$  alkenyl,  $(C_2-C_8)$  alkynyl, benzyl, aryl,  $(C_0-C_4)$  alkyl  $(C_1-C_6)$  heterocycloalkyl,  $(C_0-C_4)$  alkyl- $(C_2-C_5)$  heteroaryl,  $C(O)R^3$ ,  $C(S)R^3$ ,  $C(O)OR^5$ ,  $(C_1-C_8)$  alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$  alkyl- $OR^5$ ,  $(C_1-C_8)$  alkyl- $C(O)OR^5$ ,  $C(O)NHR^3$ ,  $C(S)NHR^3$ ,  $C(O)NR^3R^3$ ,  $C(S)NR^3R^3$  or  $(C_1-C_8)$  alkyl- $O(CO)R^5$ ;

$R^2$  is H, F, benzyl,  $(C_1-C_8)$  alkyl,  $(C_2-C_8)$  alkenyl, or  $(C_2-C_8)$  alkynyl;

$R^3$  and  $R^{3'}$  are independently  $(C_1-C_8)$  alkyl,  $(C_3-C_7)$  cycloalkyl,  $(C_2-C_8)$  alkenyl,  $(C_2-C_8)$  alkynyl, benzyl, aryl,  $(C_0-C_4)$  alkyl- $(C_1-C_6)$  heterocycloalkyl,  $(C_0-C_4)$  alkyl- $(C_2-C_5)$  heteroaryl,  $(C_0-C_8)$  alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$  alkyl- $OR^5$ ,  $(C_1-C_8)$  alkyl- $C(O)OR^5$ ,  $(C_1-C_8)$  alkyl- $O(CO)R^5$ , or  $C(O)OR^5$ ;

$R^4$  is  $(C_1-C_8)$  alkyl,  $(C_2-C_8)$  alkenyl,  $(C_2-C_8)$  alkynyl,  $(C_1-C_4)$  alkyl- $OR^5$ , benzyl, aryl,  $(C_0-C_4)$  alkyl- $(C_1-C_6)$  heterocycloalkyl, or  $(C_0-C_4)$  alkyl- $(C_2-C_5)$  heteroaryl;

$R^5$  is  $(C_1-C_8)$  alkyl,  $(C_2-C_8)$  alkenyl,  $(C_2-C_8)$  alkynyl, benzyl, aryl, or  $(C_2-C_5)$  heteroaryl;

each occurrence of  $R^6$  is independently H,  $(C_1-C_8)$  alkyl,  $(C_2-C_8)$  alkenyl,  $(C_2-C_8)$  alkynyl, benzyl, aryl,  $(C_2-C_5)$  heteroaryl, or  $(C_0-C_8)$  alkyl- $C(O)O-R^5$  or the  $R^6$  groups can join to form a heterocycloalkyl group;

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n is 0 or 1; and

\* represents a chiral-carbon center.

In specific compounds of formula II, when n is 0 then  $R^1$  is  $(C_3-C_7)$  cycloalkyl,  $(C_2-C_8)$  alkenyl,  $(C_2-C_8)$  alkynyl, benzyl, aryl,  $(C_0-C_4)$  alkyl- $(C_1-C_6)$  heterocycloalkyl,  $(C_0-C_4)$  alkyl- $(C_2-C_5)$  heteroaryl,  $C(O)R^3$ ,  $C(O)OR^4$ ,  $(C_1-C_8)$  alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$  alkyl- $OR^5$ ,  $(C_1-C_8)$  alkyl- $C(O)OR^5$ ,  $C(S)NHR^3$ , or  $(C_1-C_8)$  alkyl- $O(CO)R^5$ ;

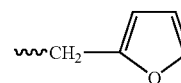
$R^2$  is H or  $(C_1-C_8)$  alkyl; and

$R^3$  is  $(C_1-C_8)$  alkyl,  $(C_3-C_7)$  cycloalkyl,  $(C_2-C_8)$  alkenyl,  $(C_2-C_8)$  alkynyl, benzyl, aryl  $(C_0-C_4)$  alkyl- $(C_1-C_6)$  heterocycloalkyl,  $(C_0-C_4)$  alkyl- $(C_2-C_5)$  heteroaryl,  $(C_5-C_8)$  alkyl- $N(R^6)_2$ ;  $(C_0-C_8)$  alkyl- $NH-C(O)O-R^5$ ;  $(C_1-C_8)$  alkyl- $OR^5$ ,  $(C_1-C_8)$  alkyl- $C(O)OR^5$ ,  $(C_1-C_8)$  alkyl- $O(CO)R^5$ , or  $C(O)OR^5$ ; and the other variables have the same definitions.

In other specific compounds of formula II,  $R^2$  is H or  $(C_1-C_4)$  alkyl.

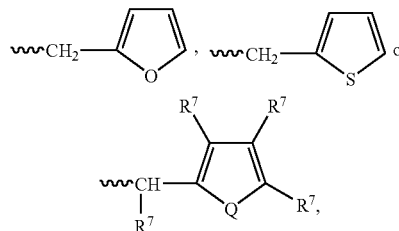
In other specific compounds of formula II,  $R^1$  is  $(C_1-C_8)$  alkyl or benzyl.

In other specific compounds of formula II,  $R^1$  is H,  $(C_1-C_8)$  alkyl, benzyl,  $CH_2OCH_3$ ,  $CH_2CH_2OCH_3$ , or



II

In another embodiment of the compounds of formula II,  $R^1$  is



wherein Q is O or S, and each occurrence of  $R^7$  is independently H,  $(C_1-C_8)$  alkyl, benzyl,  $CH_2OCH_3$ , or  $CH_2CH_2OCH_3$ .

In other specific compounds of formula II,  $R^1$  is  $C(O)R^3$ .

In other specific compounds of formula II,  $R^3$  is  $(C_0-C_4)$  alkyl- $(C_2-C_5)$  heteroaryl,  $(C_1-C_8)$  alkyl, aryl, or  $(C_0-C_4)$  alkyl- $OR^5$ .

In other specific compounds of formula II, heteroaryl is pyridyl, furyl, or thienyl.

In other specific compounds of formula II,  $R^1$  is  $C(O)OR^4$ .

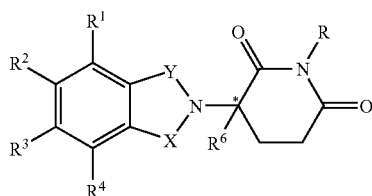
In other specific compounds of formula II, the H of  $C(O)NHC(O)$  can be replaced with  $(C_1-C_4)$  alkyl, aryl, or benzyl.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. No. 09/781,179, International Publication No. WO 98/54170, and U.S. Pat. No. 6,395,754,

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each of which are incorporated herein by reference. Representative compounds are of formula III:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C≡O;  
R is H or CH<sub>2</sub>OCOR<sup>1</sup>;

(i) each of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup>, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> is nitro or —NHR<sup>5</sup> and the remaining of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> are hydrogen;

R<sup>5</sup> is hydrogen or alkyl of 1 to 8 carbons

R<sup>6</sup> hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro;

R<sup>7</sup> is R<sup>7</sup>—CHR<sup>10</sup>—N(R<sup>8</sup>R<sup>9</sup>);

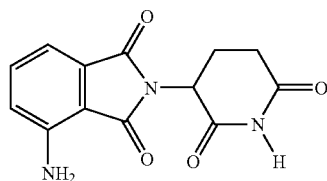
R<sup>7</sup> is m-phenylene or p-phenylene or —(C<sub>n</sub>H<sub>2n</sub>)— in which n has a value of 0 to 4;

each of R<sup>8</sup> and R<sup>9</sup> taken independently of the other is hydrogen or alkyl of 1 to 8 carbon atoms, or R<sup>8</sup> and R<sup>9</sup> taken together are tetramethylene, pentamethylene, hexamethylene, or —CH<sub>2</sub>CH<sub>2</sub>[X]X<sub>1</sub>CH<sub>2</sub>CH<sub>2</sub>— in which [X]X<sub>1</sub> is —O—, —S—, or —NH—;

R<sup>10</sup> is hydrogen, alkyl of 1 to 8 carbon atoms, or phenyl; and

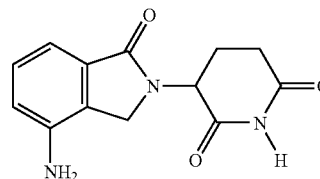
\* represents a chiral-carbon center.

The most preferred immunomodulatory compounds of the invention are 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. The compounds can be obtained via standard, synthetic methods (see e.g., U.S. Pat. No. 5,635,517, incorporated herein by reference). The compounds are available from Celgene Corporation, Warren, N.J. 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (ACTIMID™) has the following chemical structure:



3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (REVIMID™) has the following chemical structure:

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The compounds of the invention can either be commercially purchased or prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry techniques.

As used herein and unless otherwise indicated, the term “pharmaceutically acceptable salt” encompasses non-toxic acid and base addition salts of the compound to which the term refers. Acceptable non-toxic acid addition salts include those derived from organic and inorganic acids or bases known in the art, which include, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embolic acid, enanthic acid, and the like.

Compounds that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds are those that form non-toxic base addition salts, i.e., salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), lysine, and procaine.

As used herein and unless otherwise indicated, the term “prodrug” means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of immunomodulatory compounds of the invention that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of immunomodulatory compounds of the invention that comprise —NO, —NO<sub>2</sub>, —ONO, or —ONO<sub>2</sub> moieties. Prodrugs can typically be prepared using well-known methods, such as those described in 1 *Burger's Medicinal Chemistry and Drug Discovery*, 172–178, 949–982 (Manfred E. Wolff ed., 5th ed. 1995), and *Design of Prodrugs* (H. Bundgaard ed., Elsevier, N.Y. 1985).

As used herein and unless otherwise indicated, the terms “biohydrolyzable amide,” “biohydrolyzable ester,” “biohydrolyzable carbamate,” “biohydrolyzable carbonate,” “biohydrolyzable ureide,” “biohydrolyzable phosphate” mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere



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with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxymethyl, acetoxylethyl, aminocarbonyloxymethyl, pivaloyloxymethyl, and pivaloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyloxymethyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides,  $\alpha$ -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

#### 4.2. Second Active Agents

One or more second active ingredients can be used in the methods and compositions of the invention together with an immunomodulatory compound of the invention. In a preferred embodiment, the second active agents are capable of affecting or improving the process of blood cell production. Specific second active agents also stimulate the division and differentiation of committed erythroid progenitors in cells in vitro or in vivo.

Second active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules). The second active agents include but are not limited to hematopoietic growth factors, cytokines, anti-cancer agents, antibiotics, proteasome inhibitors, immunosuppressive agents and other therapeutics discussed herein. Particular agents include, but are not limited to, G-CSF, GM-CSF, EPO, dexamethasone, topotecan, pentoxifylline, irinotecan, ciprofloxacin, vinorelbine, IL2, IL8, IL18, Ara-C, isotretinoin, 13-cis-retinoic acid, 12-O-tetradecanoylphorbol-13-acetate (TPA), 5-AZA2'-deoxycytidine, 9-nitrocamp-tothecin, transretinoic acid, amifostine, amphotericin B and liposomal amphotericin B, anti-CD-20 monoclonal antibody, anti-thymocyte globulin (ATG), arsenic trioxide, azacytidine, bevacizumab, bismuth monoclonal antibody, bryostatin, busulfan, caspofungin acetate, celastrol, cladribine, cyclophosphamide, cyclosporine, cytarabine, cytosine, daunorubicin, depsipeptide, etoposide, flaresy transferase inhibitor, flavopiridol, Flt3 ligand, fludarabine, gentuzumab ozogomicin (mylotarg), etanercept (Enbrel®), imatinib (Glivec®), anti-TNF- $\alpha$  antibodies, infliximab (Remicade®), humanized monoclonal anti-VEGF antibody, idarubicin, leucovorin, melphalan, mitoxantrone, monoclonal antibody ABX-CBL, monoclonal antibody CD52, mycophenolate mofetil, oblimersen, omega-3 fatty acids, pentostatin, phenylbutyrate, PR1 leukemia peptide vaccine, montanide, proteasome inhibitor, sodium phenyl-butyrate, sodium salicylate, temozolomide, thymoglobulin, troxatyl, tumor necrosis factor receptor IgG chimera, Yttrium Y 90 humanized monoclonal antibody

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M195. In a specific embodiment of the invention, an immunomodulatory compound of the invention is used in combination with pentoxifylline, ciprofloxacin, and/or dexamethasone.

This invention also encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term "mutants" are proteins that lack carbohydrate moieties normally present in their naturally occurring forms (e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001).

Recombinant and mutated forms of G-CSF can be prepared as described in U.S. Pat. Nos. 4,810,643; 4,999,291; 5,528,823; and 5,580,755; all of which are incorporated herein by reference. Recombinant and mutated forms of GM-CSF can be prepared as described in U.S. Pat. Nos. 5,391,485; 5,393,870; and 5,229,496; all of which are incorporated herein by reference. In fact, recombinant forms of G-CSF and GM-CSF are currently sold in the United States for the treatment of symptoms associated with specific chemotherapies. A recombinant form of G-CSF known as filgrastim is sold in the United States under the trade name NEUPOGEN®. NEUPOGEN® is known to stimulate division and maturation of granulocytes, mostly neutrophils, in MDS patients and to enhance erythroid response in combination with EPO. *Physicians' Desk Reference*, 587-592 (56<sup>th</sup> ed., 2002). A recombinant form of GM-CSF known as sargramostim is also sold in the United States under the trade name LEUKINE®. LEUKINE® is known to stimulate division and maturation of earlier myeloid and macrophage precursor cells and has been reported to increase granulocytes. *Physicians' Desk Reference*, 1755-1760 (56<sup>th</sup> ed., 2002). A recombinant form of EPO known as epoetin alfa is sold in the United States under the trade name EPOGEN®. EPOGEN® is used to stimulate red cell production by stimulating division and maturation of committed red cell precursor cells. EPOGEN® has been reported to be effective in 20-26% of MDS patient when administered by itself and in as many as 48% of patients when combined with G-CSF or GM-CSF. *Physicians' Desk Reference*, 582-587 (56<sup>th</sup> ed., 2002).

A growth-factor or cytokine such as G-CSF, GM-CSF and EPO can also be administered in the form of a vaccine. For example, vaccines that secrete, or cause the secretion of, cytokines such as G-CSF and GM-CSF can be used in the methods, pharmaceutical compositions, and kits of the invention. See, e.g., Emens, L. A., et al., *Curr. Opinion Mol. Ther.* 3(1):77-84 (2001).

Other compounds that can be administered or used in combination with an immunomodulatory compound of the invention include those disclosed in U.S. provisional patent application No. 60/380,842, filed May 17, 2002, and U.S. provisional patent application No. 60/380,843, filed May 17, 2002, both of which are incorporated herein by reference.

#### 4.3. Methods of Treatment and Management

Methods of this invention encompass methods of preventing, treating and/or managing various types of MDS. As

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used herein, unless otherwise specified, the term “preventing” includes but is not limited to, inhibition or the averting of symptoms associated with MDS. The symptoms associated with MDS include, but are not limited to, anemia, thrombocytopenia, neutropenia, cytopenia, bicytopenia (two deficient cell lines), and pancytopenia (three deficient cell lines). As used herein, unless otherwise specified, the term “treating” refers to the administration of a composition after the onset of symptoms of MDS, whereas “preventing” refers to the administration prior to the onset of symptoms, particularly to patients at risk of MDS. As used herein and unless otherwise indicated, the term “managing” encompasses preventing the recurrence of MDS in a patient who had suffered from MDS, lengthening the time a patient who had suffered from MDS remains in remission, and/or preventing the occurrence of MDS in patients at risk of suffering from MDS.

The invention encompasses methods of treating or preventing patients with primary and secondary MDS. It further encompasses methods treating patients who have been previously treated for MDS, as well as those who have not previously been treated for MDS. Because patients with MDS have heterogenous clinical manifestations and varying clinical outcomes, it has become apparent that staging the patients according to their prognosis and approaching therapy depending on the severity and stage is necessary. Indeed, the methods and compositions of this invention can be used in various stages of treatments for patients with one or more types of MDS including, but not limited to, refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess blasts (RAEB), RAEB in transformation (RAEB-T), or chronic myelomonocytic leukemia (CMML). The invention also contemplates treating patients diagnosed using the IPSS for MDS discussed above. Greenberg et al., *Blood* 1997 (89):2079–88.

Methods encompassed by this invention comprise administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof to a patient (e.g., a human) suffering, or likely to suffer, from MDS. Specific patient populations include the elderly, i.e., ages 60 and above as well as those over 35 years of age. Patients with familial history of MDS or leukemia are also preferred candidates for preventive regimens.

In one embodiment of the invention, an immunomodulatory compound of the invention is administered orally and in a single or divided daily doses in an amount of from about 0.10 to about 150 mg/day. In a particular embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) is administered in an amount of from about 0.1 to about 1 mg per day, or alternatively about 5 mg every other day. 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) can be preferably administered in an amount of from about 5 to 25 mg per day, or alternatively from about 25 to about 50 mg every other day.

#### 4.3.1 Combination Therapy With A Second Active Agent

Particular methods of the invention comprise administering 1) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and 2) a second active agent or active ingredient. Examples of immunomodulatory compounds of the invention are disclosed herein (see, e.g., section 4.1); and examples of the second active agents are also disclosed herein (see, e.g., section 4.2).

Administration of the immunomodulatory compounds and the second active agents to a patient can occur simul-

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taneously or sequentially by the same or different routes of administration. The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. A preferred route of administration for an immunomodulatory compound is oral. Preferred routes of administration for the second active agents or ingredients of the invention are known to those of ordinary skill in the art. See, e.g., *Physicians' Desk Reference*, 1755–1760 (56<sup>th</sup> ed., 2002).

In one embodiment, the second active agent is administered intravenously or subcutaneously and once or twice daily in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. The specific amount of the second active agent will depend on the specific agent used, the type of MDS being treated or managed, the severity and stage of MDS, and the amount(s) of immunomodulatory compounds of the invention and any optional additional active agents concurrently administered to the patient. In a particular embodiment, the second active agent is GM-CSF, G-CSF, EPO, transretinoic acid, dexamethasone, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, or a combination thereof. GM-CSF is administered in an amount of from about 60 to about 500 mcg/m<sup>2</sup> intravenously over 2 hours, or from about 5 to about 12 mcg/m<sup>2</sup>/day subcutaneously. G-CSF is administered subcutaneously in an amount of about 1 mcg/kg/day initially and can be adjusted depending on rise of total granulocyte counts. The maintenance dose is 300 (in smaller patients) or 480 mcg subcutaneously. EPO is administered subcutaneously in an amount of 10,000 Unit 3 times per week.

#### 4.3.2 Use With Transplantation Therapy

In still another embodiment, this invention encompasses a method of treating, preventing and/or managing MDS, which comprises administering the immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with transplantation therapy. As discussed elsewhere herein, the treatment of MDS is based on the stages and mechanism of the disease. As inevitable leukemic transformation develops in certain stages of MDS, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. The combined use of the immunomodulatory compound of the invention and transplantation therapy provides a unique and unexpected synergism. In particular, an immunomodulatory compound of the invention exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy in patients with MDS. An immunomodulatory compound of the invention can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of related Graft Versus Host Disease (GVHD). This invention encompasses a method of treating, preventing and/or managing MDS which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after the transplantation of umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow. Examples of stem cells suitable for use in the methods of the invention are disclosed in U.S.

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provisional patent application No. 60/372,348, filed Apr. 12, 2002 by R. Hariri et al., the entirety of which is incorporated herein by reference.

#### 4.3.3. Cycling Therapy

In certain embodiments, the prophylactic or therapeutic agents of the invention are cyclically administered to a patient. Cycling therapy involves the administration of a first agent for a period of time, followed by the administration of the agent and/or the second agent for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

In a particular embodiment, prophylactic or therapeutic agents are administered in a cycle of about 16 weeks, about once or twice every day. One cycle can comprise the administration of a therapeutic or prophylactic agent and at least one (1) or three (3) weeks of rest. The number of cycles administered is from about 1 to about 12 cycles, more typically from about 2 to about 10 cycles, and more typically from about 2 to about 8 cycles.

#### 4.4. Pharmaceutical Compositions and Single Unit Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Pharmaceutical compositions and dosage forms of the invention can further comprise one or more excipients.

Pharmaceutical compositions and dosage forms of the invention can also comprise one or more additional active ingredients. Consequently, pharmaceutical compositions and dosage forms of the invention comprise the active ingredients disclosed herein (e.g., an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second active ingredient). Examples of optional additional active ingredients are disclosed herein (see, e.g., section 4.2).

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), or parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), transdermal or transcutaneous administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease. These and other

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ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients may be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose other mono- or di-saccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the *U.S. Pharmacopeia* (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not



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limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of from about 0.10 to about 150 mg. Typical dosage forms comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of about 0.1, 1, 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 50, 100, 150 or 200 mg. In a particular embodiment, a preferred dosage form comprises 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) in an amount of about 1, 2, 5, 10, 25 or 50 mg. In a specific embodiment, a preferred dosage form comprises 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) in an amount of about 5, 10, 25 or 50 mg. Typical dosage forms comprise the second active ingredient in an amount of 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. Of course, the specific amount of the second active ingredient will depend on the specific agent used, the type of MDS being treated or managed, and the amount(s) of immunomodulatory compounds of the invention, and any optional additional active agents concurrently administered to the patient.

#### 4.4.1 Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or non-aqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly

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and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. An specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil,

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light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

A preferred solid oral dosage form of the invention comprises an immunomodulatory compound of the invention, anhydrous lactose, microcrystalline cellulose, polyvinylpyrrolidone, stearic acid, colloidal anhydrous silica, and gelatin.

#### 4.4.2 Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

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#### 4.4.3 Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention. For example, cyclodextrin and its derivatives can be used to increase the solubility of an immunomodulatory compound of the invention, and its derivatives. See, e.g., U.S. Pat. No. 5,134,127, which is incorporated herein by reference.

#### 4.4.4 Topical and Mucosal Dosage Forms

Topical and mucosal dosage forms of the invention include, but are not limited to, sprays, aerosols, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide topical and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form solutions, emulsions or gels, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990).

The pH of a pharmaceutical composition or dosage form may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this

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regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

## 4.4.5 Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a dosage form of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, prodrug, or clathrate thereof. Kits encompassed by this invention can further comprise additional active ingredients such as G-CSF, GM-CSF, EPO, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13-cis-retinoic acid, or a pharmacologically active mutant or derivative thereof, or a combination thereof. Examples of the additional active ingredients include, but are not limited to, those disclosed herein (see, e.g., section 4.2).

Kits of the invention can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

## 5. EXAMPLES

The following studies are intended to further illustrate the invention without limiting its scope.

Excessive production of the growth inhibitory cytokine TNF- $\alpha$  is demonstrated in bone marrow plasma of patients with MDS, implicating TNF- $\alpha$  as a critical negative regulator of erythroid progenitor survival in the disorder. As a result, a study with an immunomodulatory compound of the invention was conducted.

## 5.1. Pharmacology and Toxicology Studies

A series of non-clinical pharmacology and toxicology studies have been performed to support the clinical evaluation of an immunomodulatory compound of the invention in human subjects. These studies were performed in accordance with internationally recognized guidelines for study design and in compliance with the requirements of Good Laboratory Practice (GLP), unless otherwise noted.

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The pharmacological properties of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, including activity comparisons with thalidomide, have been characterized in *in vitro* studies. Studies examined the effects of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione or thalidomide on the production of various cytokines. In all studies, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione was at least 50 times more potent than thalidomide. In addition, a safety pharmacology study of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione has been conducted in dogs and the effects of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione on ECG parameters were examined further as part of three repeat-dose toxicity studies in primates. The results of these studies are described below.

## 5.2. Modulation of Cytokine Production

Inhibition of TNF- $\alpha$  production following LPS-stimulation of human PBMC and human whole blood by 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione or thalidomide was investigated *in vitro* (Muller et al., *Bioorg. Med. Chem. Lett.* 9:1625-1630, 1999). The  $IC_{50}$ 's of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and human whole blood were ~100 nM (25.9 ng/mL) and ~480 nM (103.6 ng/mL), respectively. Thalidomide, in contrast, had an  $IC_{50}$  of ~194  $\mu$ M (50.2  $\mu$ g/mL) for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC.

*In vitro* studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but 50 to 2000 times more potent than, thalidomide. The pharmacological effects of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione derive from its action as an inhibitor of cellular response to receptor-initiated trophic signals (e.g., IGF-1, VEGF, cyclooxygenase-2), and other activities. As a result, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione suppresses the generation of inflammatory cytokines, down-regulates adhesion molecules and apoptosis inhibitory proteins (e.g., cFLIP, cIAP), promotes sensitivity to death-receptor initiated programmed cell death, and suppresses angiogenic response. The studies show that 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione abrogates mitogenic response to VEGF in AML cells by extinguishing ligand-induced Akt-phosphorylation, and selectively suppresses MDS vs normal bone marrow progenitor formation in pre-clinical models.

## 5.3. Clinical Studies in MDS Patients

## Protocol

An immunomodulatory compound of the invention, such as 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, is administered in an amount of from about 0.1 to about 25 mg per day to patients with MDS for 16 weeks, who are subsequently evaluated for a hematological response. Response rates are assessed in cohorts stratified by the likelihood of an MDS subtype to transform to leukemia according to the International Prognostic Scoring System (IPSS)-defined risk groups (i.e., IPSS Low and Intermediate I; versus IPSS Intermediate II and High).

For example, fifteen patients are enrolled in the first cohort and receive treatment with 25 mg per day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. The number of patients who subsequently experience an erythroid response (major or minor response) by week 16 is evaluated. If no responses are observed, the study is



terminated due to lack of efficacy. If, however, 4 or more patients respond, the study is terminated due to promising clinical activity. In the intermediate case (e.g., 1, 2 or 3 patients respond), a second cohort of 10 patients is enrolled. If after the completion of treatment by the second cohort, 4 or more patients respond among the 25 patients treated, it is concluded that the 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione shows promising clinical activity.

#### Clinical Study

Clinical studies were performed for the remitting potential of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in MDS patients with red blood cell transfusion-dependence ( $>4$  units/8 weeks) or symptomatic anemia (Hgb $<10$  g/dl). Patients received continuous treatment with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione at a oral dose of 25 mg daily. Responses were assessed according to IWG criteria after 16 weeks of treatments. Among 15 patients receiving the treatments, 11 patients were evaluable for toxicity, nine patients were evaluable for response ( $>8$  wks therapy), and three patients discontinued the therapy prematurely ( $<2$  weeks) due to cholecystitis, autoimmune hemolytic anemia, or patient refusal. Median age of the patients was 78 years ranging from 51 to 82 years. FAB types of the MDS patients include RA [4 patients], RARS [4 patients], RAEB [6 patients], and RAEB-T [1 patient] with corresponding IPSS categories of Low/Int-1 in 11 patients and Int-2/High in four patients. Myelosuppression, which was characterized by higher than grade 3 common toxicity criteria or 50% decrease in leukocyte and platelet counts [9 patients], and grade 3 fatigue [1 patient], necessitated dose reduction to 10 mg in the initial ten patients. All subsequent patients initiated oral administrations with 10 mg daily. Grade 1,2 drug-related adverse effects were limited to the 25 mg dose and included pruritus or itchy scalp [6 patients] and myalgia [1 patient]. Six (66%) of nine evaluable patients experienced hematologic benefit (dual lineage, 1 patient), including 6/9 (86%) patients with IPSS Low/Int-1. Hematologic responses included RBC transfusion-independence [4 patients], decrease in RBC transfusions of more than 50% [1 patient], increase in Hgb of more than 1.5 g [1 patient], and one minor platelet response (increase of more than 30,000/ $\mu$ L). Among five patients evaluable for cytogenetic response, three patients achieved either a complete or partial (decrease in abnormal metaphases of more than 50%) remission. Responses were associated with normalization of blast percentage [1 patient], reduced grade of BM cytologic dysplasia, and 50% to more than 40 times improvement in BM multipotent progenitor (CFU-GEMM) and erythroid burst (BFU-E) formation. Correlation with changes in apoptotic index, angiogenic features (cellular/plasma VEGF, microvessel density), cytokine generation, and proliferative fraction (Ki67) are in progress. The results of this study indicate that 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione has remarkable erythropoietic and cytogenetic remitting activity in patients with low/intermediate-1 risk MDS. Clinical benefit appears greatest in patients with low/intermediate-1 disease or the 5q-syndrome, associated with resolution of cytology dysplasia. The increase in apoptotic index, restoration of CFC, and suppression of karyotypic abnormalities suggest that the compound accelerates extinction of myelodysplastic clones. Based upon these data, the study has been expanded to treat additional subjects. Treatment with 10 mg as a continuous oral daily dose is well-tolerated with minimal myelosuppression.

#### Expanded Study

The clinical study was expanded with additional 16 MDS patients for at least eight weeks. According to the IPSS, 13 of these patients were categorized as low- or intermediate-1-risk patients and three patients were grouped as intermediate-2- or high-risk patients. According to the FAB classification, there were 11 patients with refractory anemia (RA) or RA with ringed sideroblasts (RARS), and five patients with RA with excess blasts (RAEB), RAEB in transformation (RAEB-T). The starting dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione was 25 mg daily for the first 13 patients and 10 mg daily for the remaining three patients. All patients receiving the starting dose of 25 mg required dose reduction by the completion of eight weeks therapy. Among these 16 patients who completed at least 8 weeks of monitoring, nine patients achieved an erythroid response as assessed by the International MDS Working Group Criteria. The erythroid responses consisted of transfusion independence in seven previously transfusion-dependent patients, a  $>2$  g/dL rise in blood hemoglobin concentration in one patient in with transfusion-independent anemia, and a  $>50\%$  decrease in RBC transfusion requirement in one transfusion-dependent patient. Therefore, a major erythroid response developed in eight of 16 patients and a minor erythroid response was observed in one patient. All of nine patients who showed erythroid response were low- or intermediate-1-risk patients. One patient also had a minor platelet response. In addition, complete cytogenetic responses developed in five in eight patients with abnormal karyotypes at baseline. These five patients with complete cytogenetic responses all had the Del5q31-33 abnormality, which has been discovered to be a good prognostic factor for MDS. Indeed, all five patients who enrolled in this study with 5q-syndrome achieved a complete cytogenetic response and a major erythroid response. The study also indicated an association of this therapy with an increased apoptotic index for myelodysplastic progenitors and recovery of normal hematopoietic progenitor cells.

#### 5.4. Cycling Therapy in MDS Patients

As mentioned above, immunomodulatory compounds of the invention can be cyclically administered to patients with MDS. Cycling therapy involves the administration of a first agent for a period of time, followed by the administration of the agent and/or the second agent for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

#### Example 1

In a specific embodiment, prophylactic or therapeutic agents are administered in a cycle of about 16 weeks, about once or twice every day. One cycle can comprise the administration of a therapeutic or prophylactic agent and at least one (1), two (2), or three (3) weeks of rest. The number of cycles administered is from about 1 to about 12 cycles, more typically from about 2 to about 10 cycles, and more typically from about 2 to about 8 cycles.

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## Example 2

The objectives of the study are to evaluate the efficacy and safety of oral administration of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in patients with MDS. Patients receive the compound in an amount of 10 mg/d or 15 mg/d for 21 days every 28 days in 4-week cycles for 16 weeks (4 cycles) or 24 weeks (6 cycles). The subject population comprises patients with low- or intermediate-1-risk MDS (International Prognostic Scoring System) with red blood cell transfusion-dependent anemia who have received at least two units of RBCs within 8 week of baseline (first day of study treatment). In addition to hematological laboratory monitoring, bone marrow aspirates/biopsies with cytogenetic analyses are obtained at baseline, after the completion of 3 cycles and after the completion of 6 cycles. The bone marrow, safety and efficacy data are reviewed to assess benefit-to-risk considerations throughout the study. The study reviews red blood cell transfusion independence and major erythroid response according to the International MDS Working Group Criteria. Further, the study observes red blood cell transfusion independence in the subgroup of patients with the 5q deletion cytogenetic abnormality; platelet, neutrophil, bone marrow and cytogenetic responses; and minor erythroid response of >50 % but <100 % reduction in red blood cell transfusion requirement over an 8 week period. The study further monitors adverse events, hematological tests, serum chemistries, TSH, urinalysis, urine or serum pregnancy tests, vital signs, ECG and physical examinations.

## Example 3

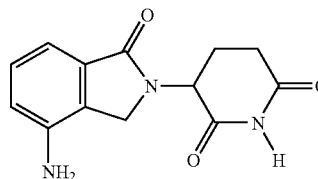
The objectives of the study are to compare the efficacy and safety of oral administration of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione to that of placebo plus standard care in patients with MDS. Patients receive the therapy in 4-week cycles for 16 weeks (4 cycles) or 24 weeks (6 cycles). The subject population comprise patients with low- or intermediate-1-risk MDS (International Prognostic Scoring System) with red blood cell transfusion-dependent anemia who have received at least two units of RBCs within 8 week of baseline (first day of study treatment). The study visits to assess safety and efficacy occur every 4 weeks and hematologic laboratory monitoring is performed every 2 weeks. Bone marrow aspirates/biopsies with cytogenetic analyses are obtained at baseline after the completion of 3 cycles and after the completion of 6 cycles. Bone marrow findings, safety and efficacy data are reviewed to assess benefit-to-risk considerations throughout the study. An extension study of continued treatments with the administration of the compound is available for patients who derive clinical benefit from 6 cycles of the therapy and to provide an opportunity for subjects who were randomized to placebo to cross over to the therapy.

Embodiments of the invention described herein are only a sampling of the scope of the invention. The full scope of the invention is better understood with reference to the attached claims.

What is claimed is:

1. A method of treating a myelodysplastic syndrome, which comprises administering to a patient in need thereof about 5 to about 50 mg per day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione having the formula:

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or a pharmaceutically acceptable salt, solvate or stereoisomer thereof.

2. The method of claim 1, wherein the compound is a pharmaceutically acceptable salt.

3. The method of claim 1, wherein the compound is a pharmaceutically acceptable solvate.

4. The method of claim 1, wherein the compound is a pharmaceutically acceptable stereoisomer.

5. The method of claim 4, wherein the stereoisomer is an enantiomerically pure R isomer.

6. The method of claim 4, wherein the stereoisomer is an enantiomerically pure S isomer.

7. The method of claim 1, which further comprises administering a therapeutically effective amount of a second active agent.

8. The method of claim 7, wherein the second active agent is capable of improving blood cell production.

9. The method of claim 7, wherein the second active agent is a cytokine, hematopoietic growth factor, an anti-cancer agent, an antibiotic, a proteasome inhibitor, or an immunosuppressive agent.

10. The method of claim 9, wherein the second active agent is etanercept, imatinib, anti-TNF- $\alpha$  antibodies, infliximab, G-CSF, GM-CSF, EPO, topotecan, pentoxifylline, ciprofloxacin, irinotecan, vinblastine, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13-cis-retinoic acid, arsenic trioxide or a pharmacologically active mutant or derivative thereof.

11. The method of claim 1, wherein the myelodysplastic syndrome is refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, or chronic myelomonocytic leukemia.

12. The method of claim 1, wherein the compound or a pharmaceutically acceptable salt, solvate or stereoisomer thereof is administered before, during or after transplanting umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow in the patient.

13. The method of claim 10, wherein the second active agent is dexamethasone.

14. The method of claim 1, wherein the patient is not previously treated for a myelodysplastic syndrome.

15. The method of claim 1, wherein the patient has been previously treated for a myelodysplastic syndrome.

16. The method of claim 1, wherein the compound is administered orally.

17. The method of claim 16, wherein the compound is administered in the form of a capsule or tablet.

18. The method of claim 1, wherein the compound is administered cyclically.

19. The method of claim 18, wherein the compound is administered once or twice every day for sixteen or twenty-four weeks.



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20. The method of claim 18, wherein one cycle comprises the administration of the compound and at least one, two, or three weeks of rest.

21. The method of claim 18, wherein the number of cycle is from one to twelve cycles.

22. The method of claim 18, wherein 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is administered in an amount of from about 5 to about 25 mg per day for 21 days every 28 days for sixteen or twenty-four weeks.

23. The method of claim 3 wherein said solvate is a hydrate.

24. The method of claim 1, wherein the compound is administered in an amount of from about 5 mg per day to about 25 mg per day.

25. The method of claim 1, wherein the compound is administered in an amount of 10 mg per day.

26. The method of claim 1, wherein the compound is administered in an amount of 15 mg per day.

27. The method of claim 1, wherein the compound is administered in an amount of 25 mg per day.

28. The method of claim 1, wherein the compound is administered in an amount of from about 25 mg every other day to about 50 mg every other day.

29. The method of claim 1, wherein the compound is administered in a cycle of about 16 weeks and about once or twice every day.

30. The method of claim 29, wherein said cycle comprises at least one (1), two (2), or three (3) weeks of rest.

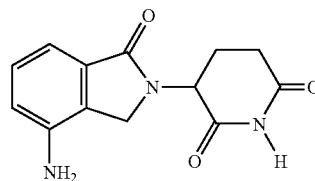
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31. The method of claim 1, wherein the compound is administered in an amount of 10 mg per day or 15 mg per day for 21 days out of a block of 28 days.

32. The method of claim 1, wherein the compound is administered orally in an amount of 5 mg as a capsule per day.

33. The method of claim 1, wherein the compound is administered orally in an amount of 10 mg as a capsule per day.

34. The method of claim 1, wherein the compound is 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, having the formula:



as a free base.

\* \* \* \* \*

# **EXHIBIT B**

US007465800B2

(12) **United States Patent**  
**Jaworsky et al.**

(10) **Patent No.:** **US 7,465,800 B2**  
(45) **Date of Patent:** **Dec. 16, 2008**

(54) **POLYMORPHIC FORMS OF  
3-(4-AMINO-1-OXO-1,3  
DIHYDRO-ISOINDOL-2-YL)-PIPERIDINE-  
2,6-DIONE**

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patent is extended or adjusted under 35  
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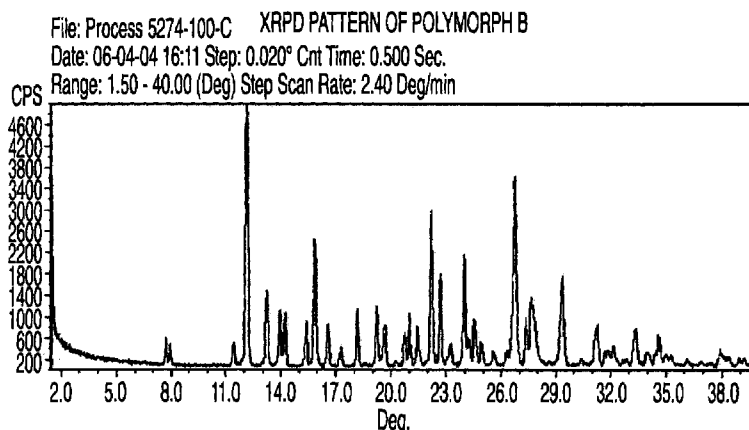
(57) **ABSTRACT**

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Polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoin-  
dol-2-yl)-piperidine-2,6-dione are disclosed. Compositions  
comprising the polymorphic forms, methods of making the  
polymorphic forms and methods of their use are also dis-  
closed.

**14 Claims, 48 Drawing Sheets**



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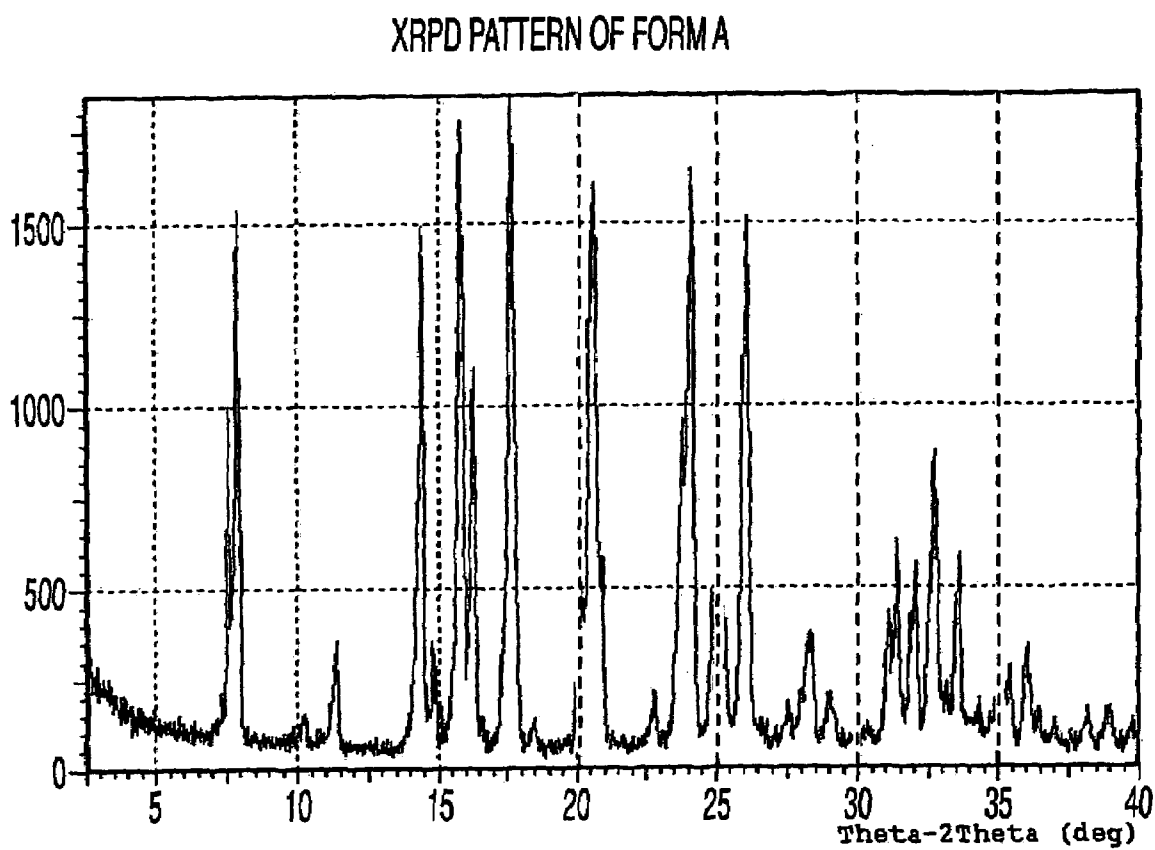
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*Fig. 1*

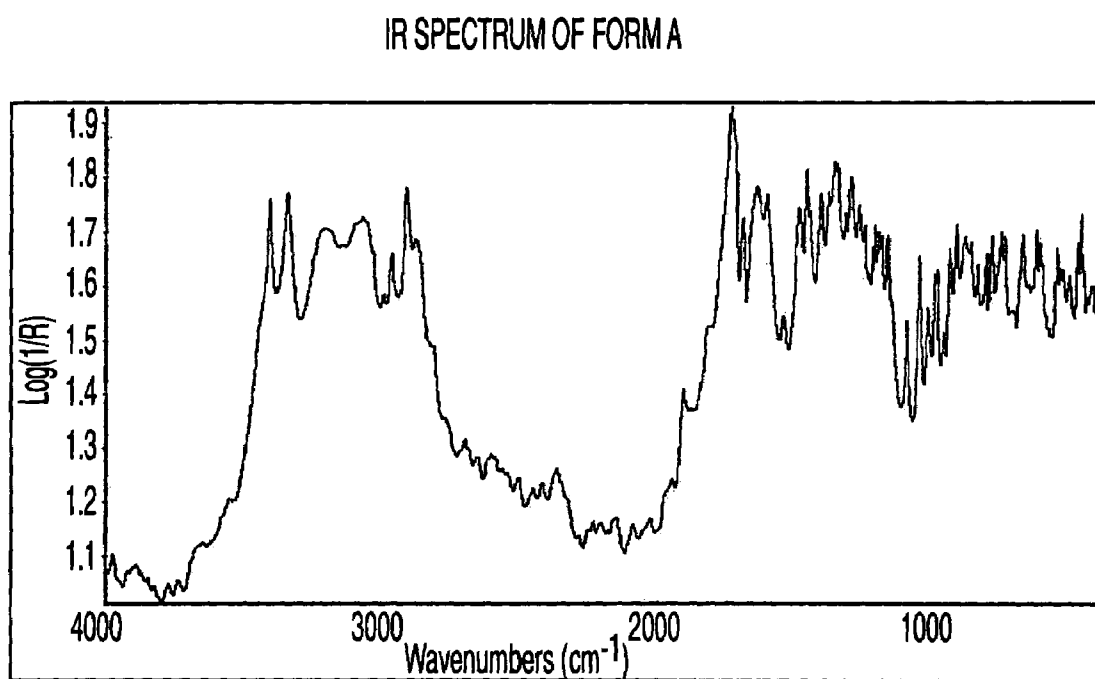


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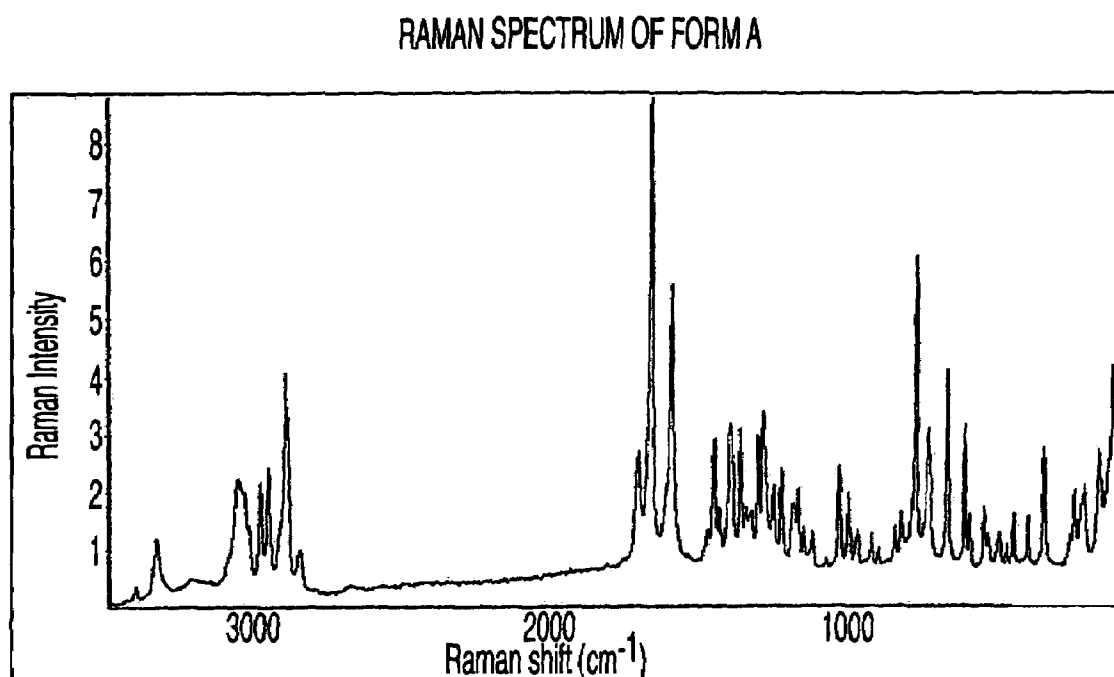
*Fig. 2*

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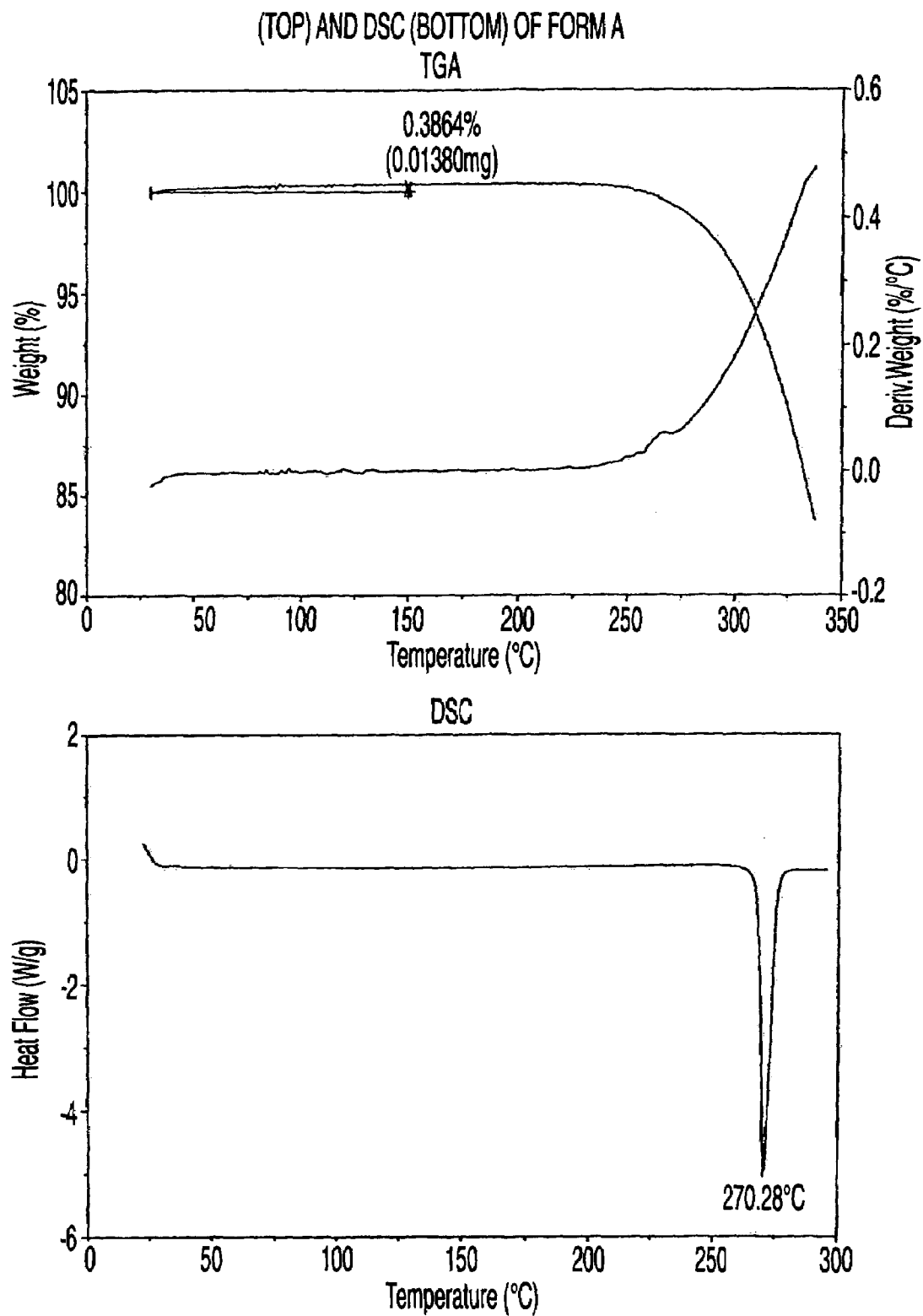
*Fig. 3*

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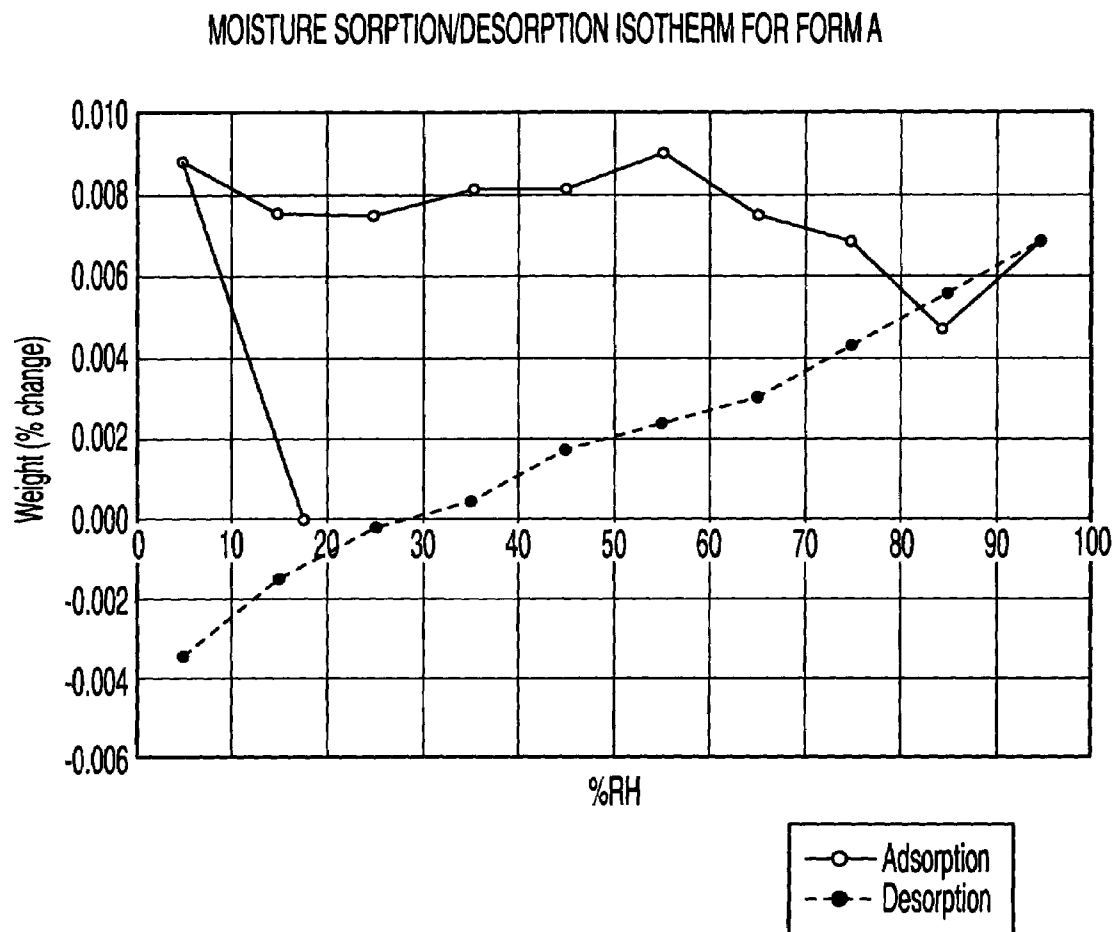
*Fig. 4*

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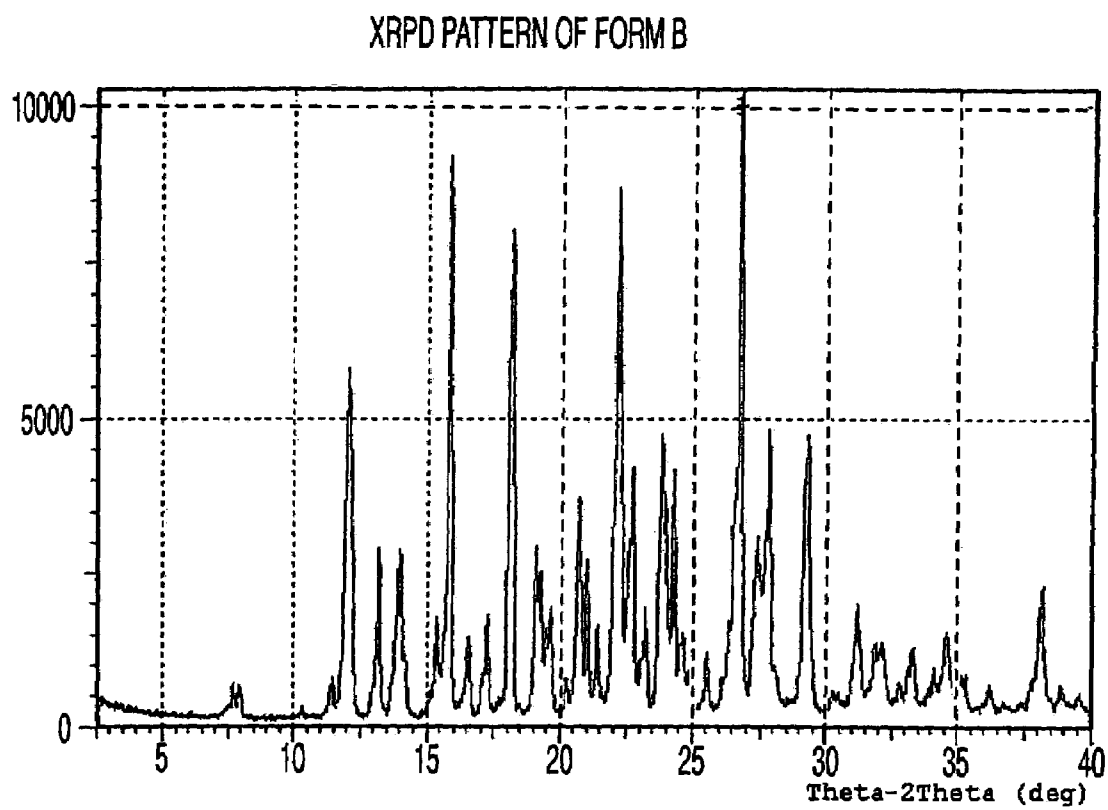


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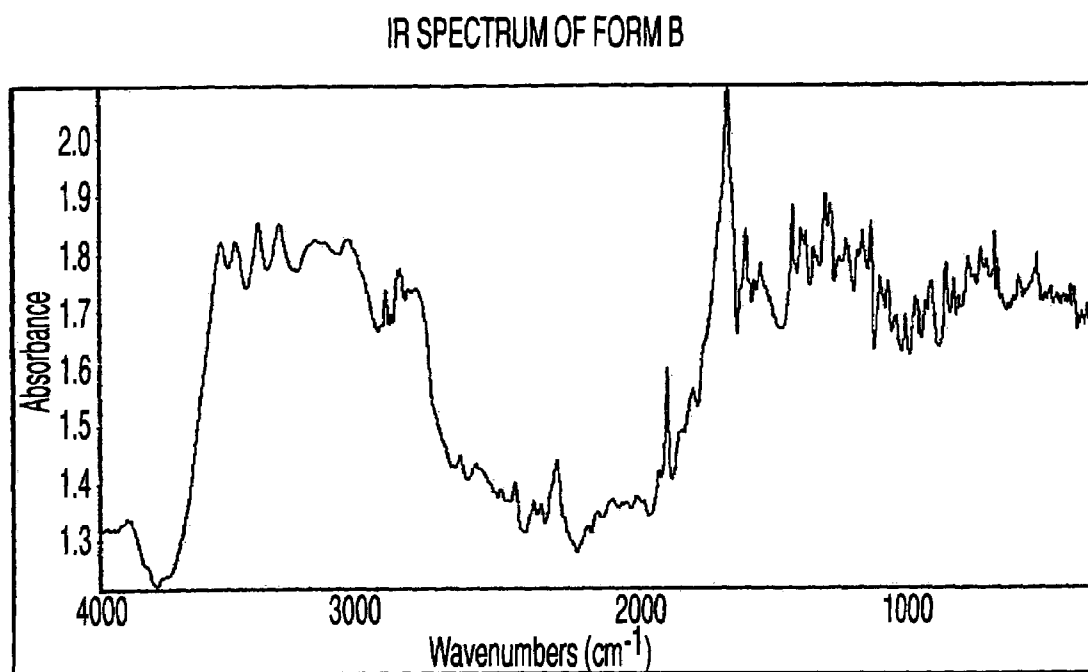
*Fig. 6*

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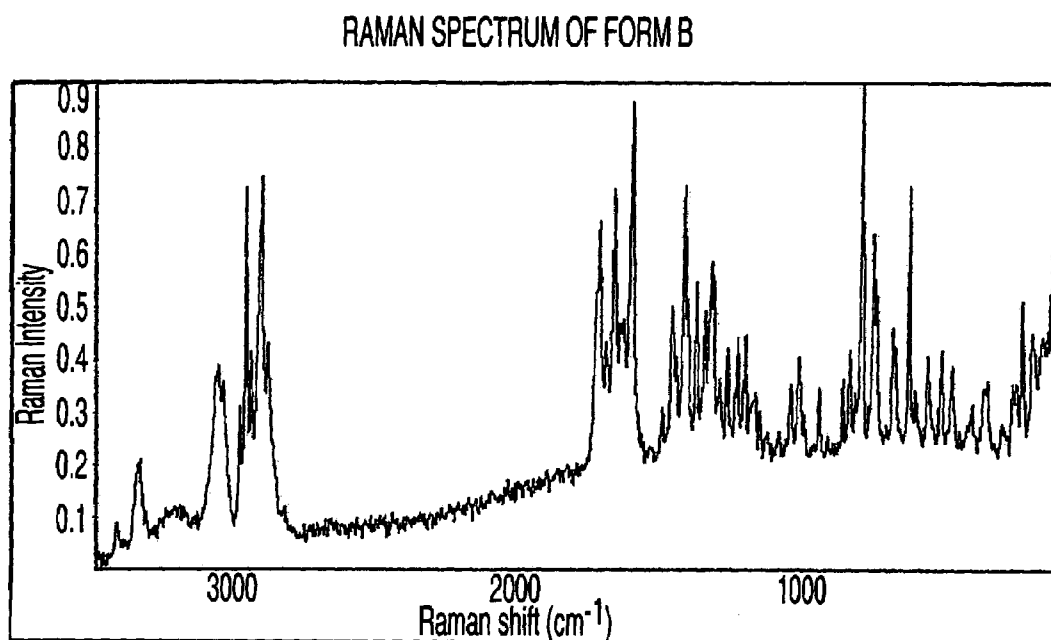
*Fig. 7*

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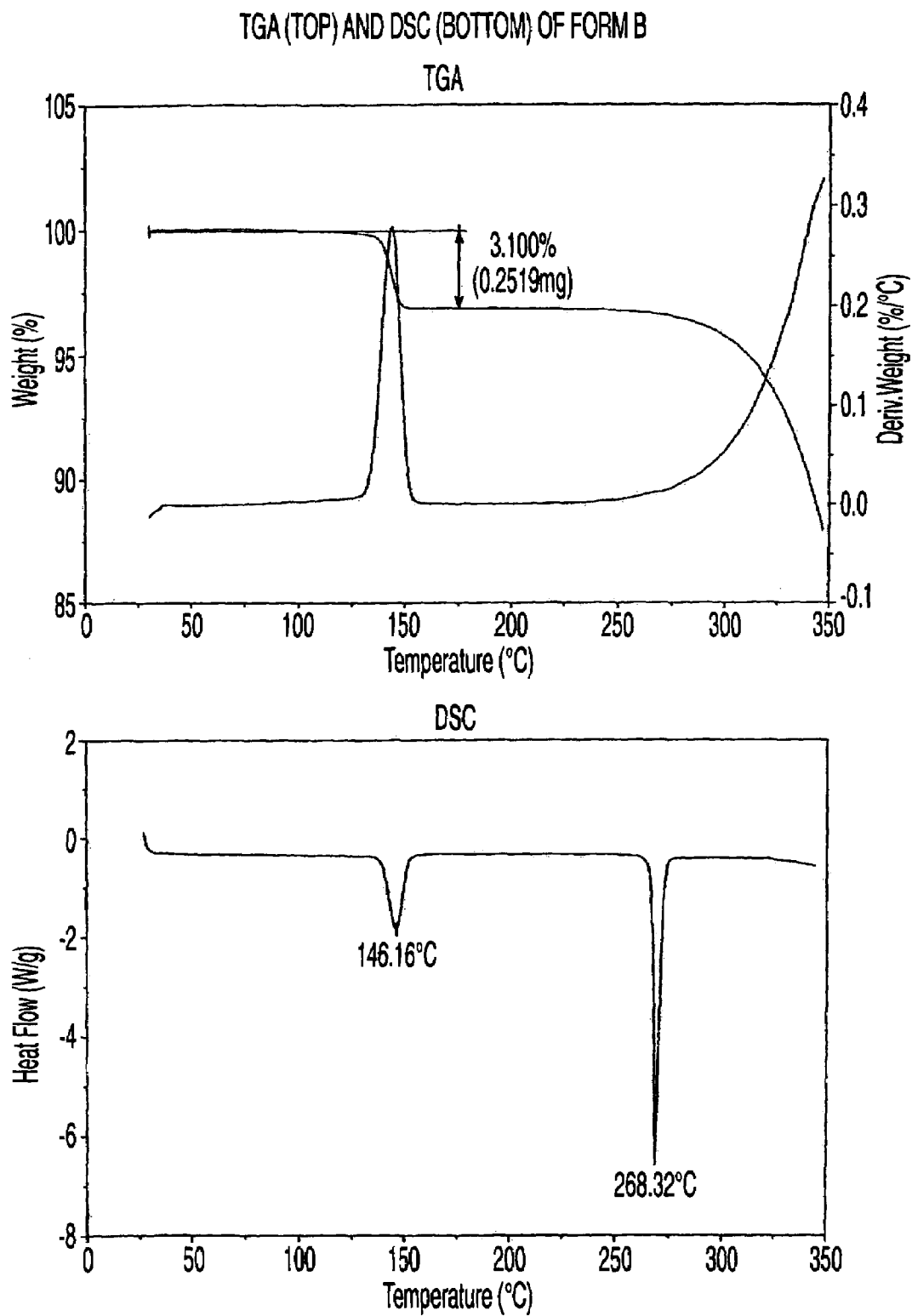
*Fig. 8*

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*Fig. 9*



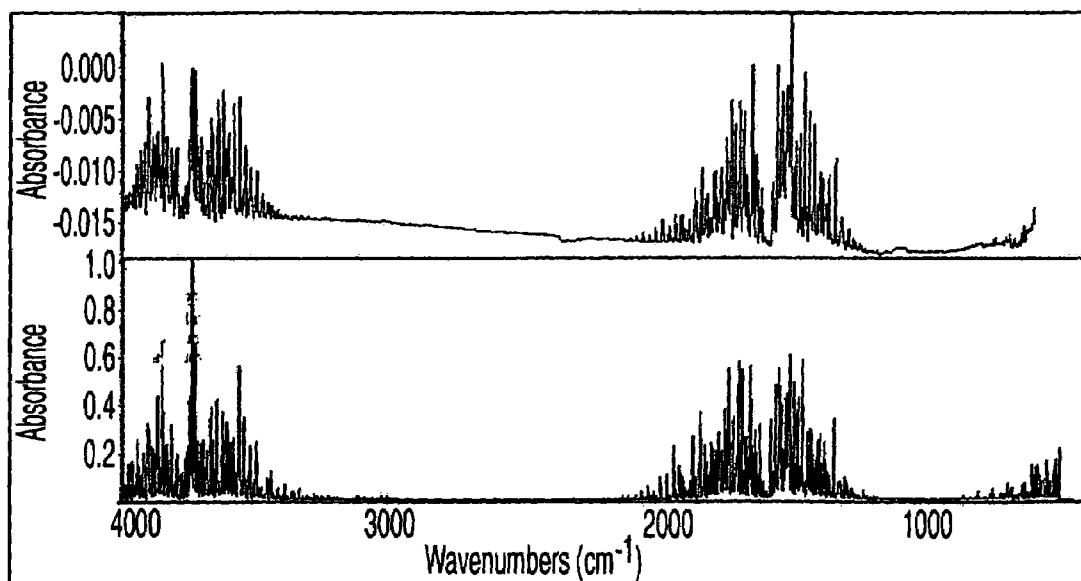
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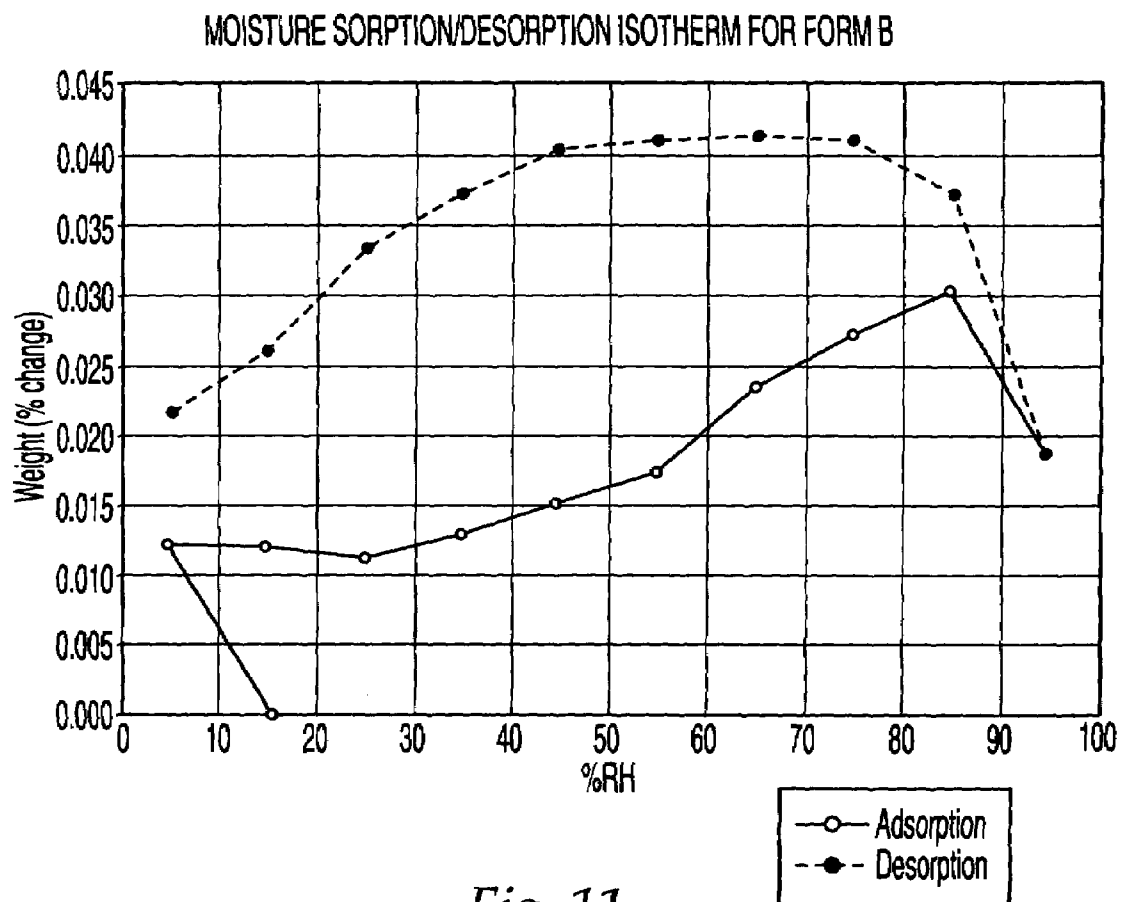
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**TG-IR RESULTS FOR FORM B**



*Fig. 10*

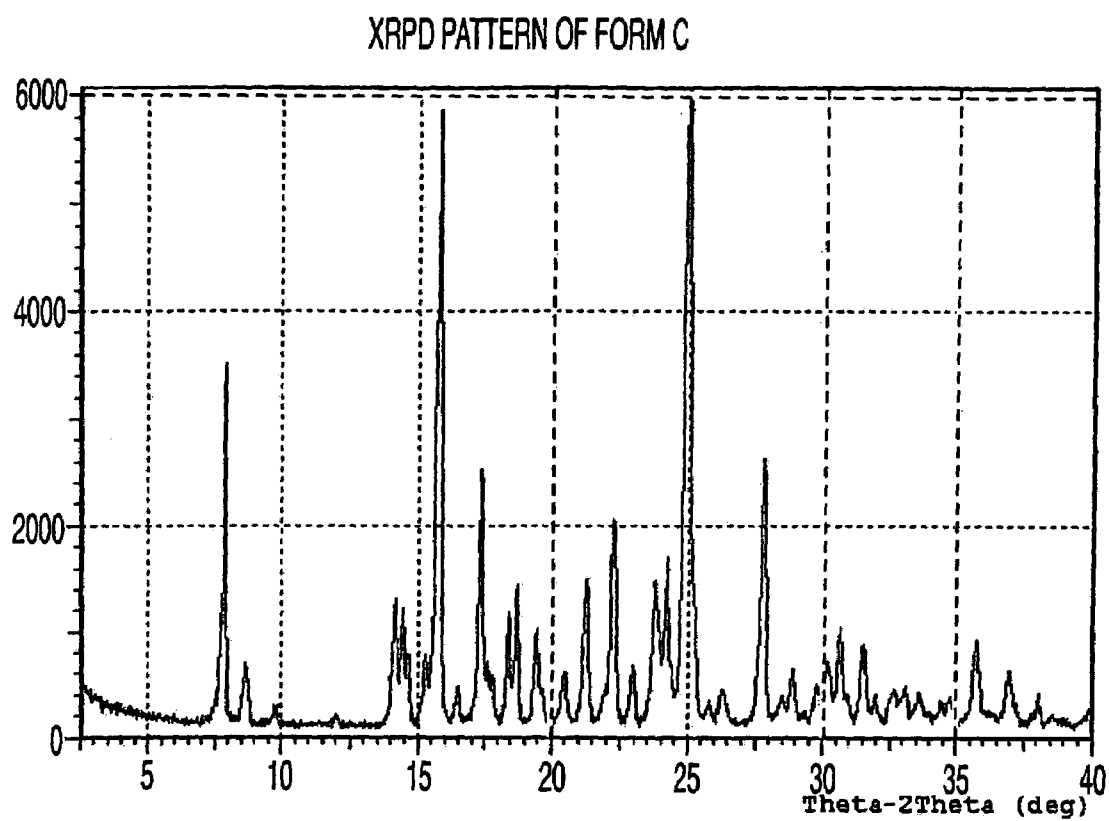


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*Fig. 12*

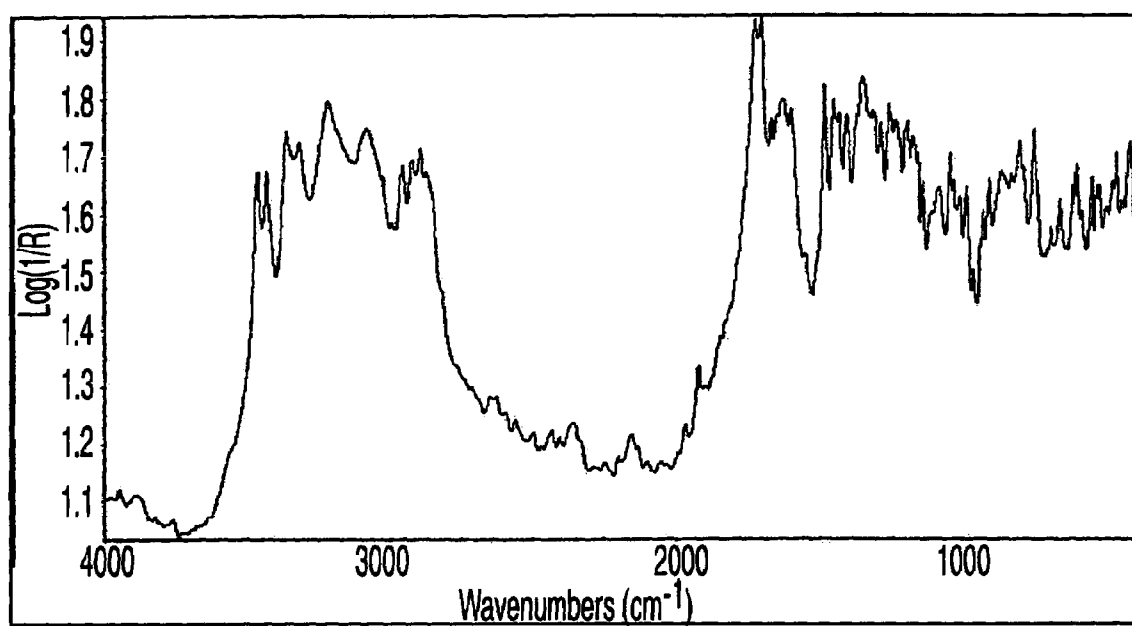
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**IR SPECTRUM OF FORM C**



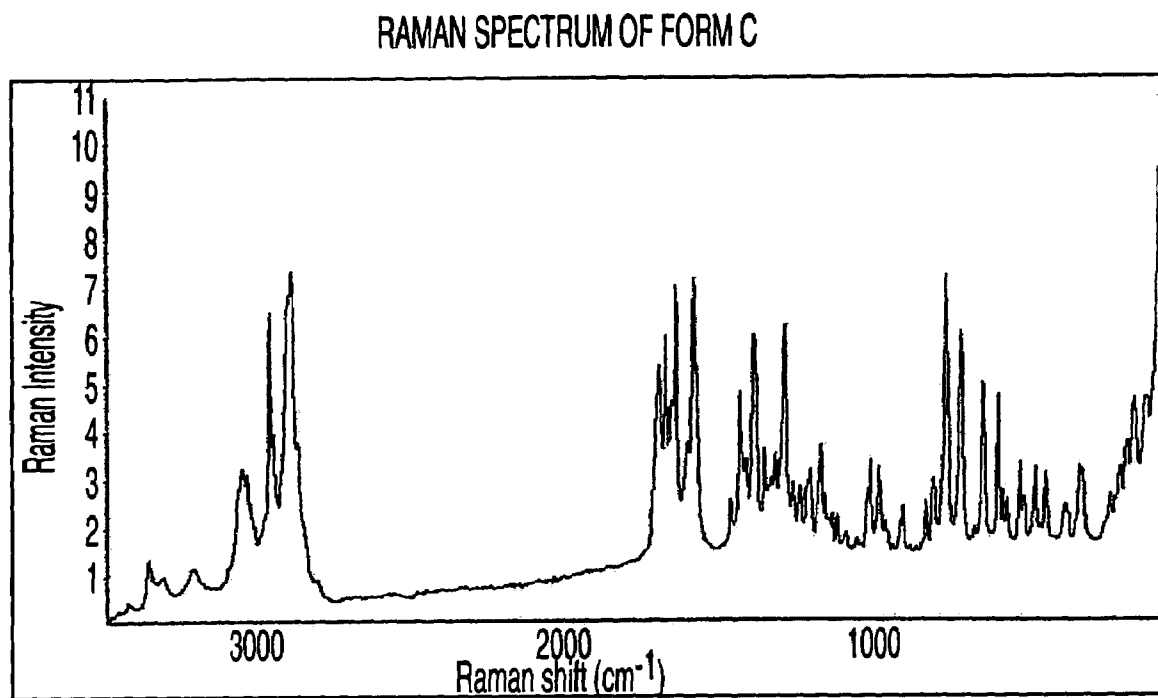
*Fig. 13*

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*Fig. 14*

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## TGA (TOP) AND DSC (BOTTOM) OF FORM C

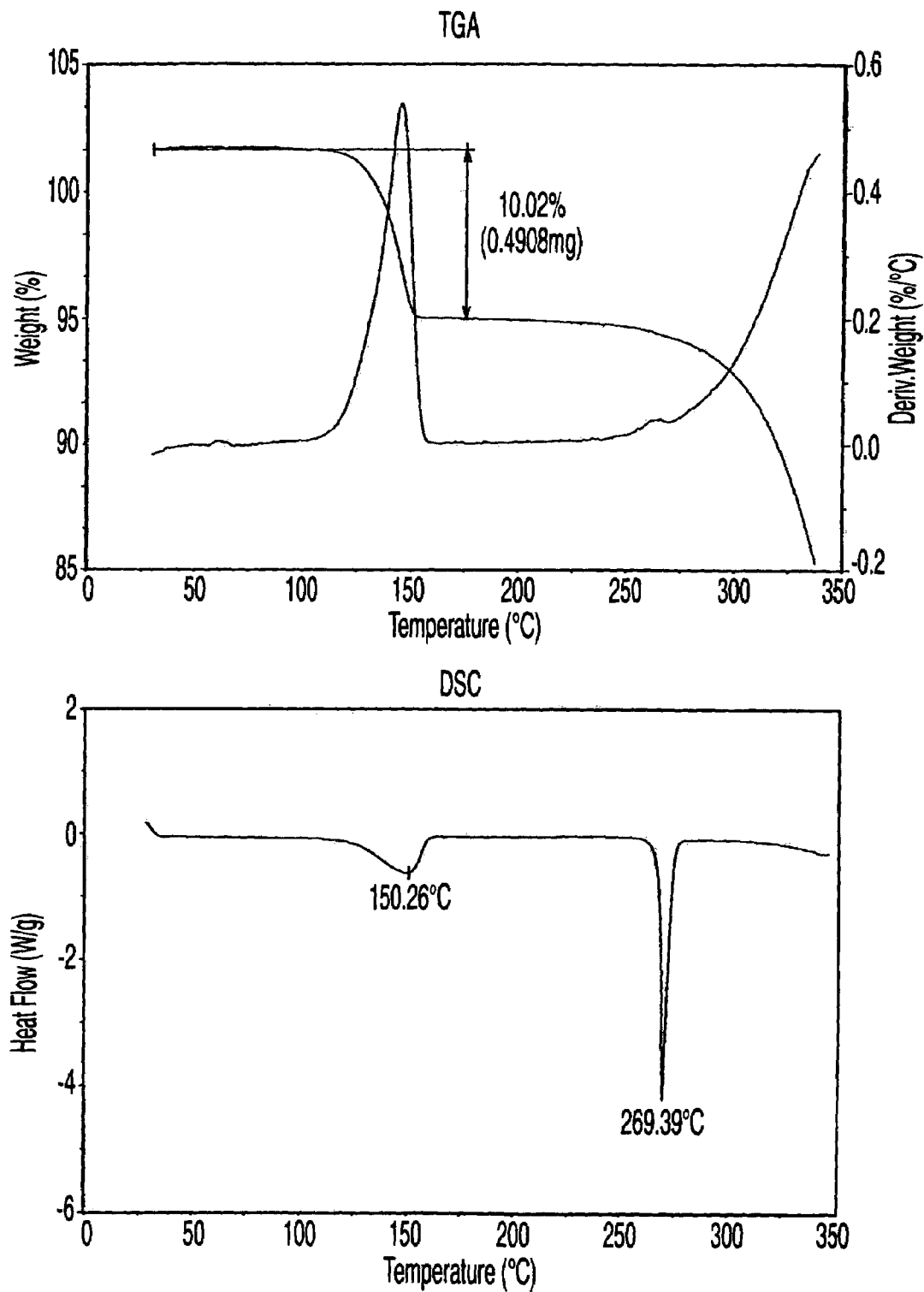


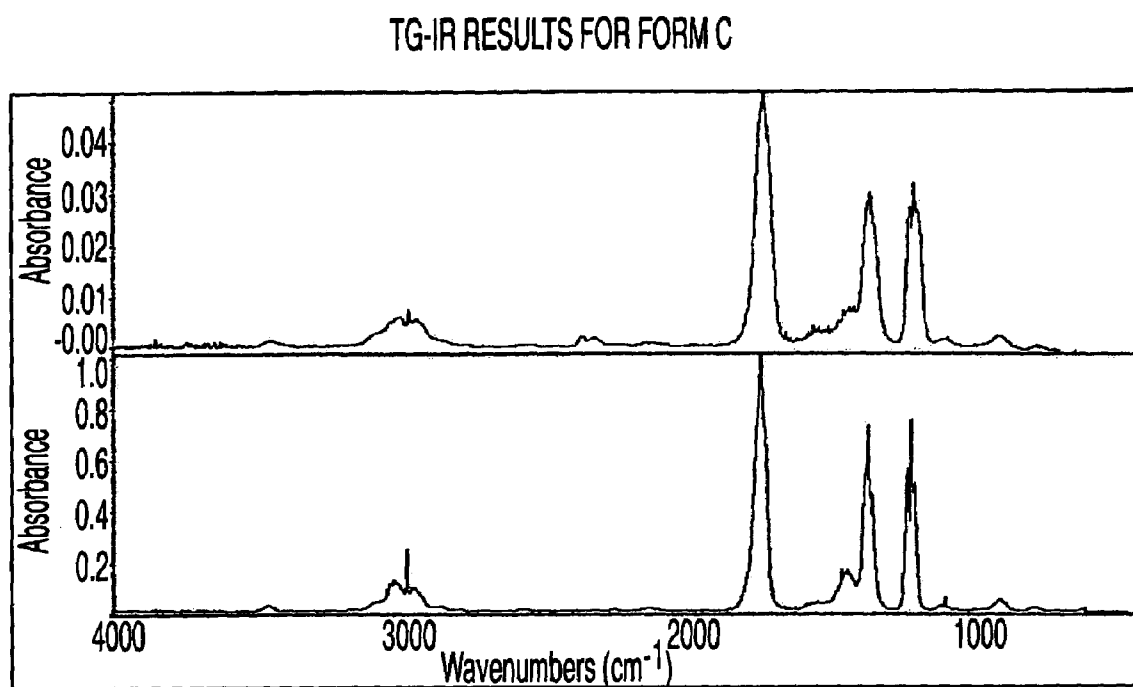
Fig. 15

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*Fig. 16*

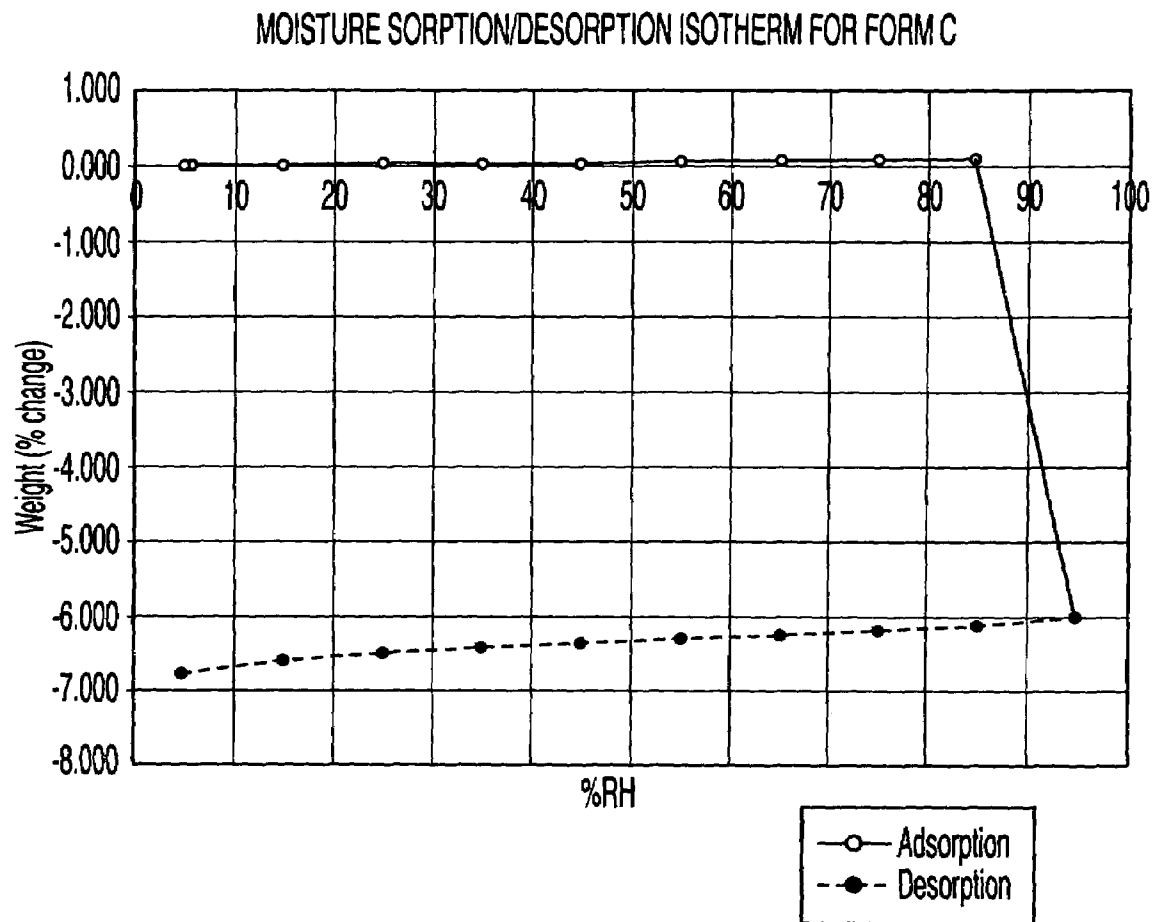


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*Fig. 17*

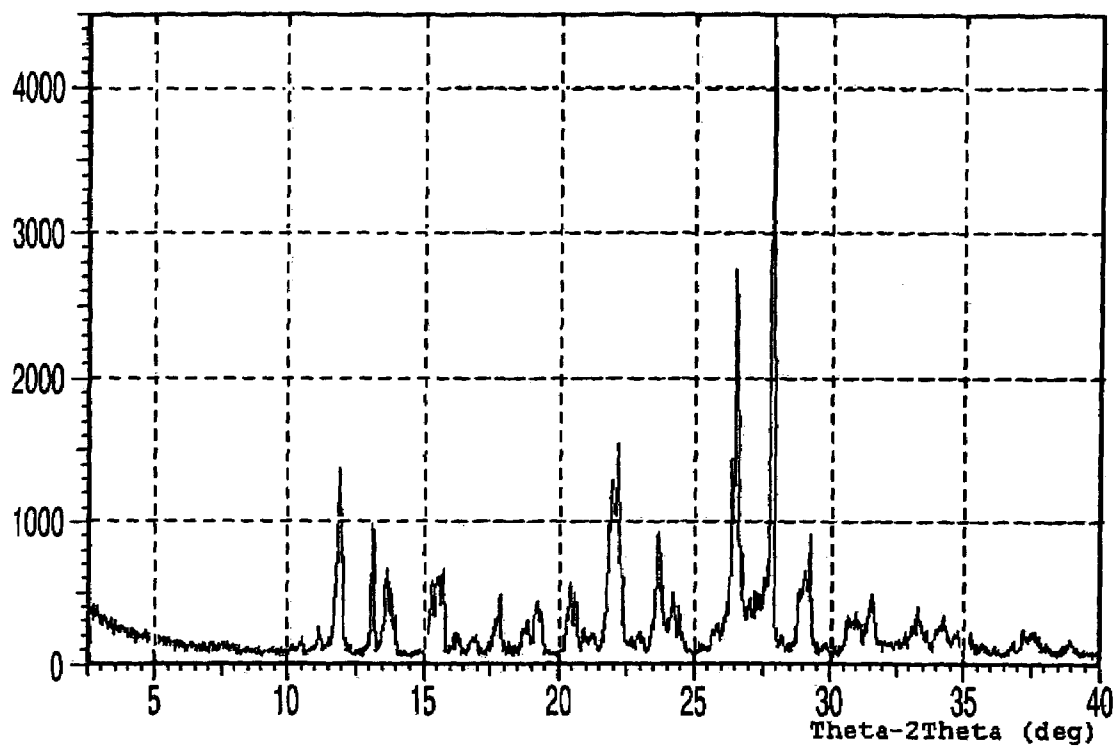
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**XRPD PATTERN OF FORM D**



*Fig. 18*

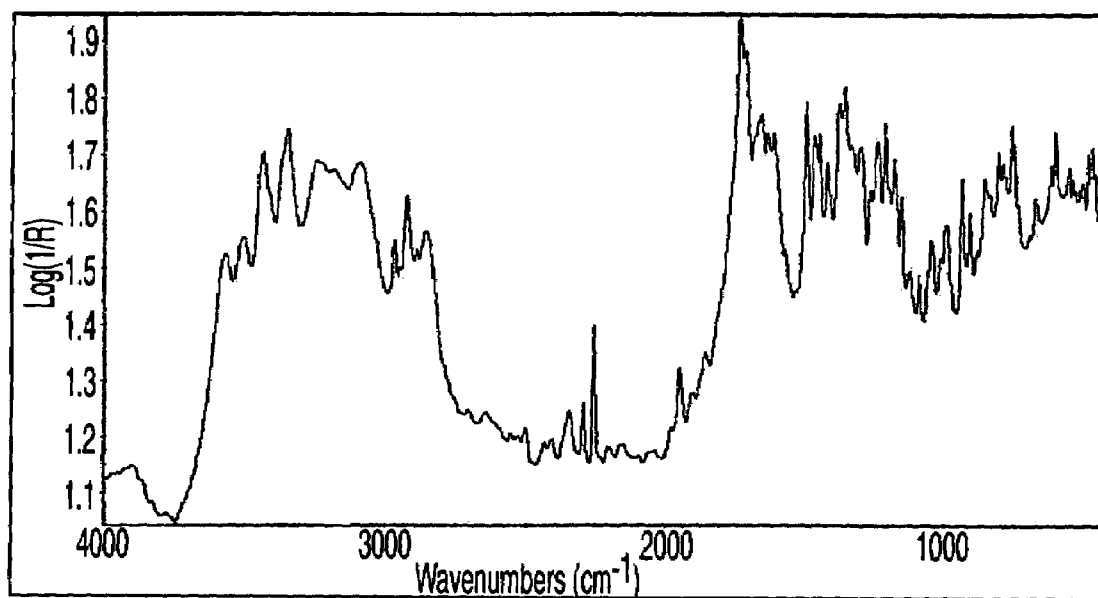
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**IR SPECTRUM OF FORM D**



*Fig. 19*

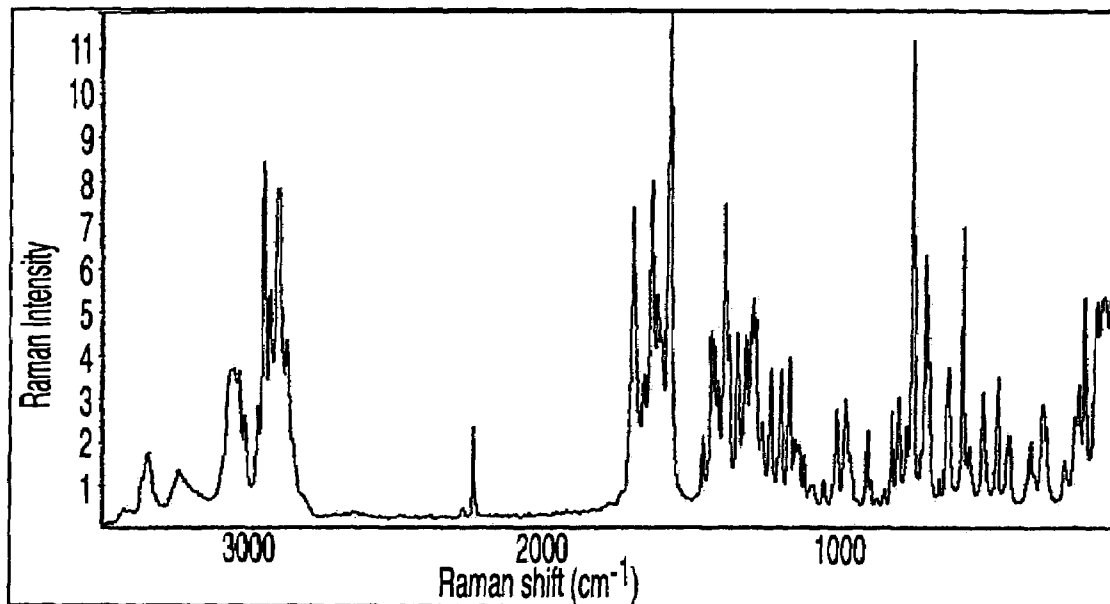
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**RAMAN SPECTRUM OF FORM D**



*Fig. 20*

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## TGA (TOP) AND DSC (BOTTOM) OF FORM D

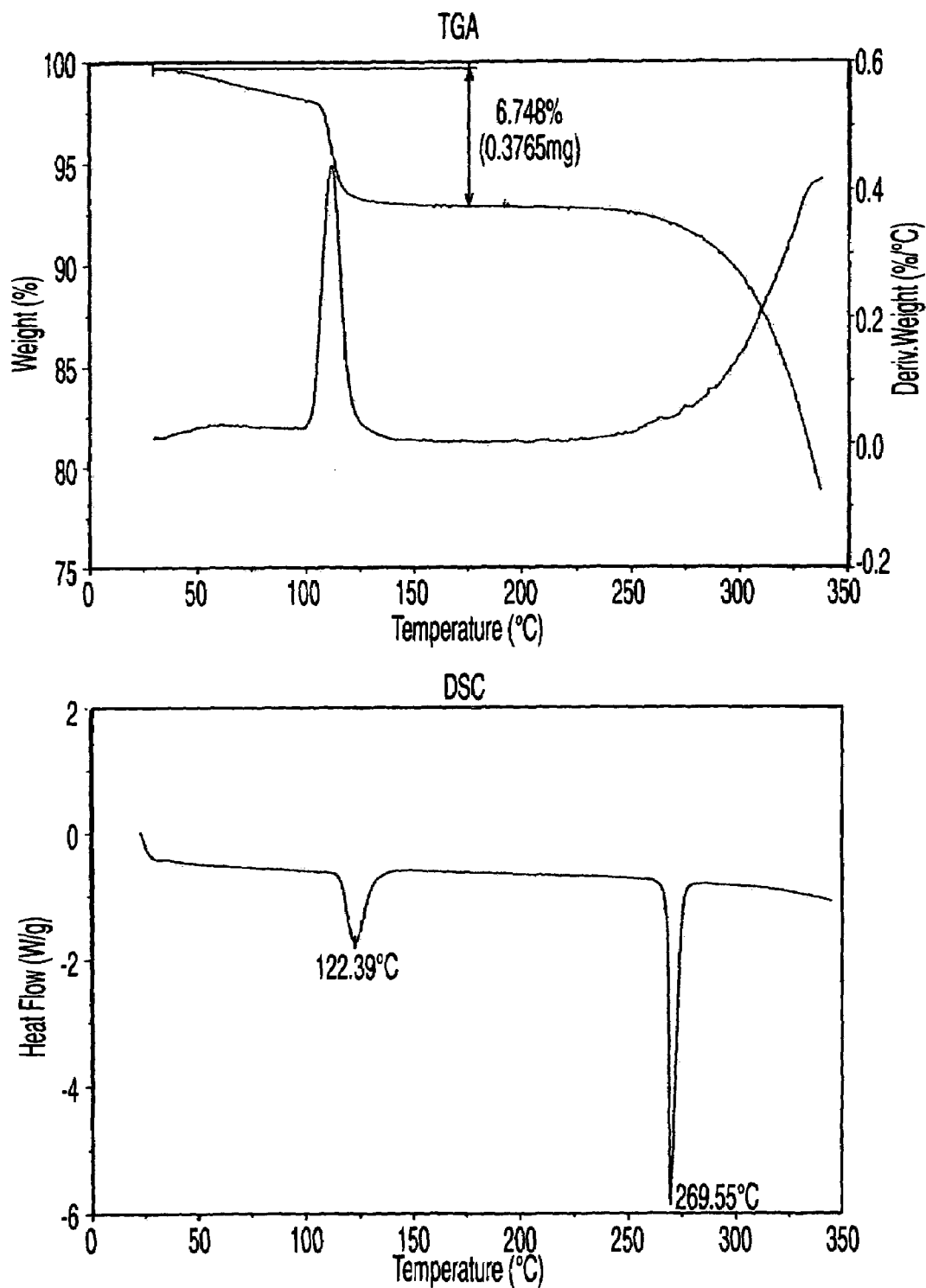


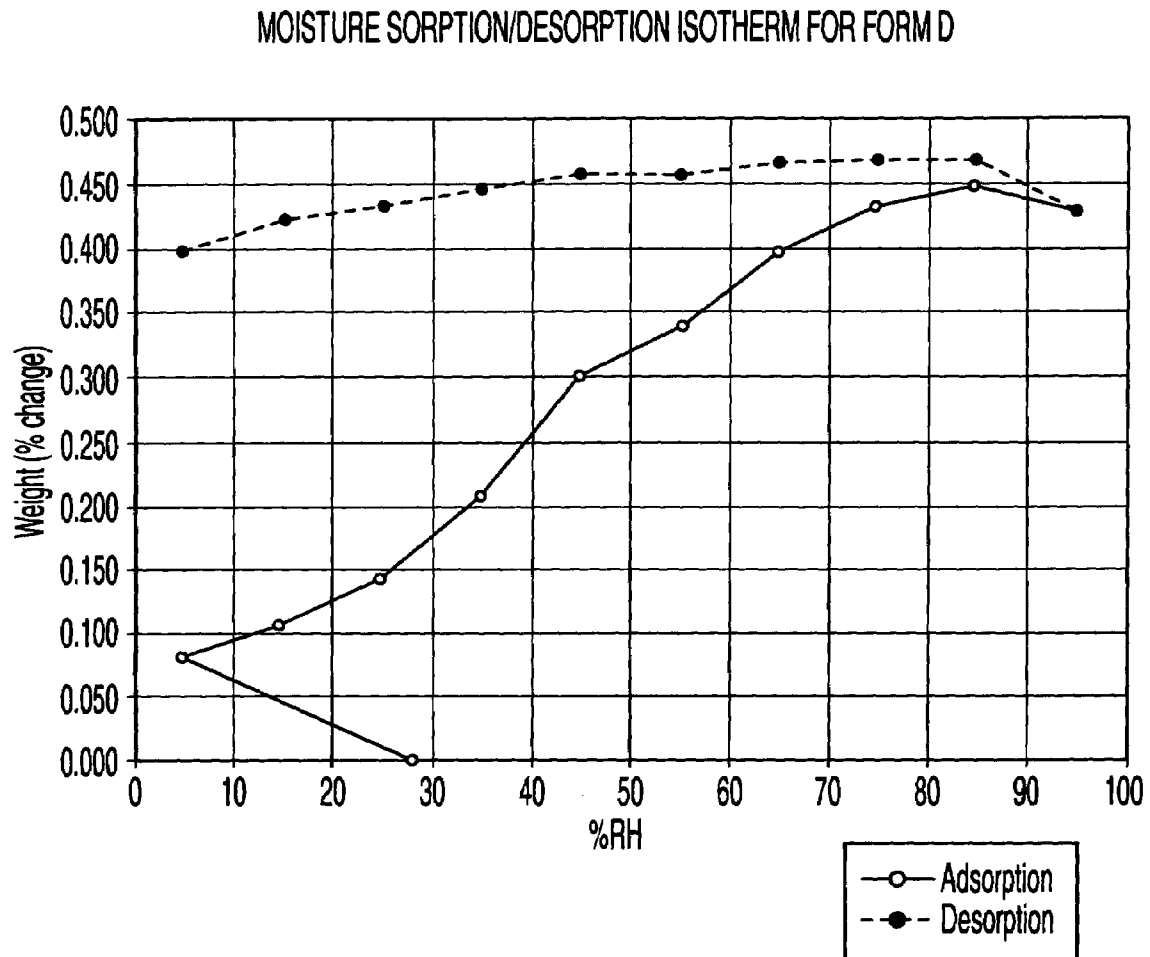
Fig. 21

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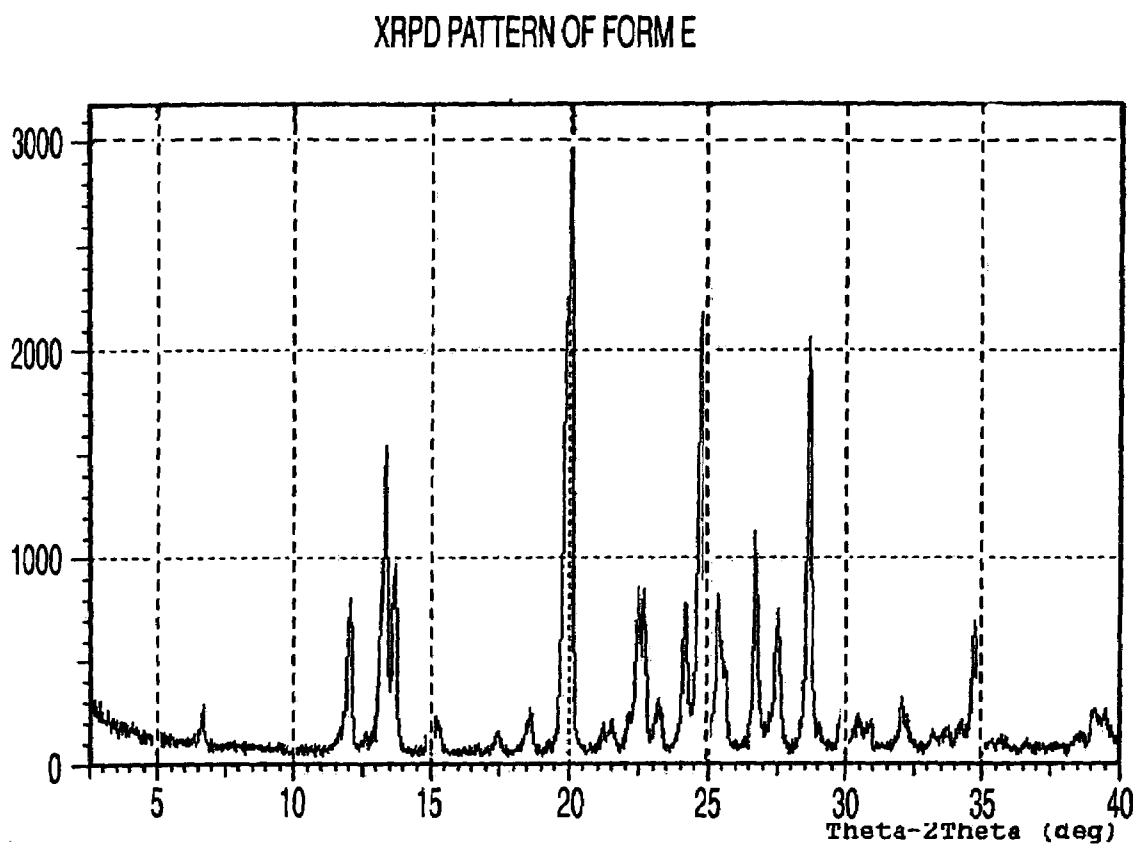
*Fig. 22*

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*Fig. 23*

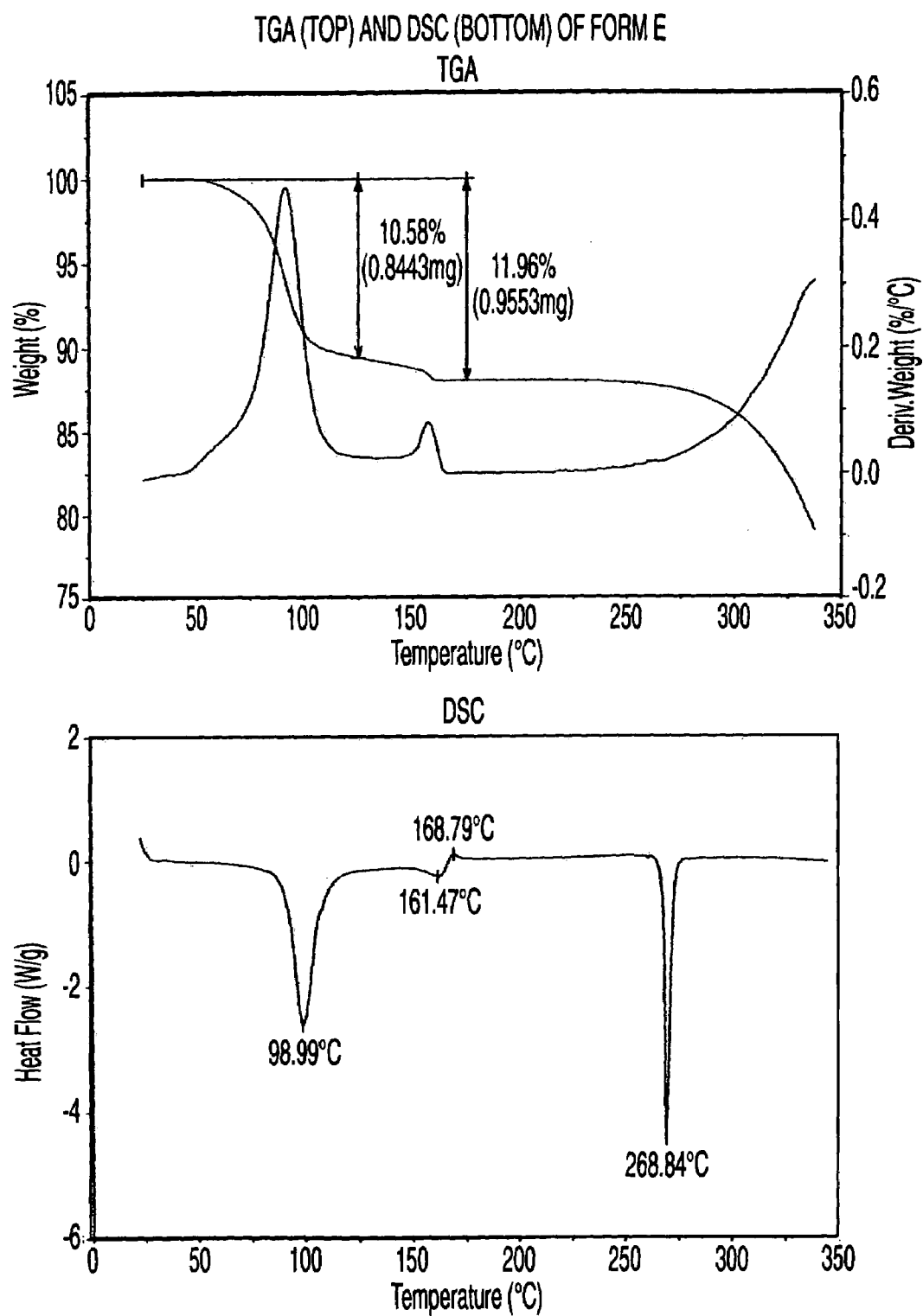


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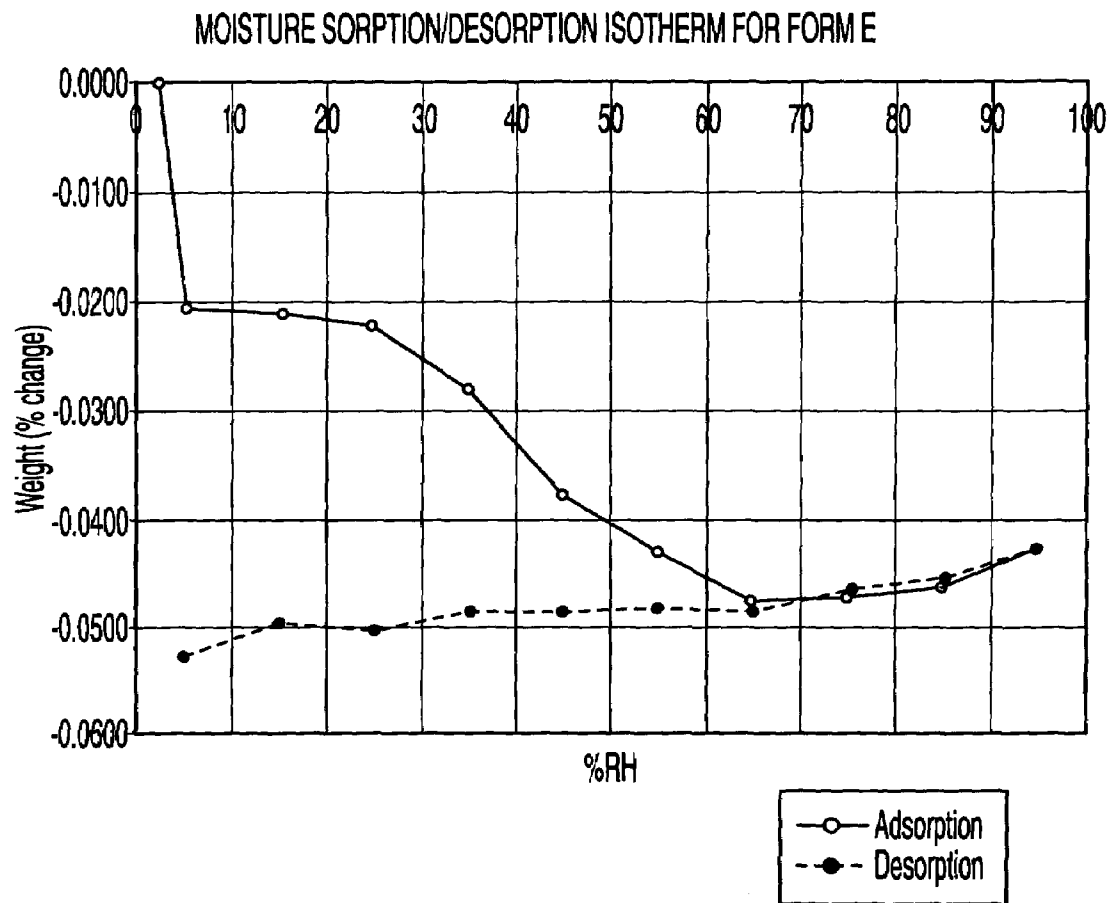
*Fig. 24*

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*Fig. 25*

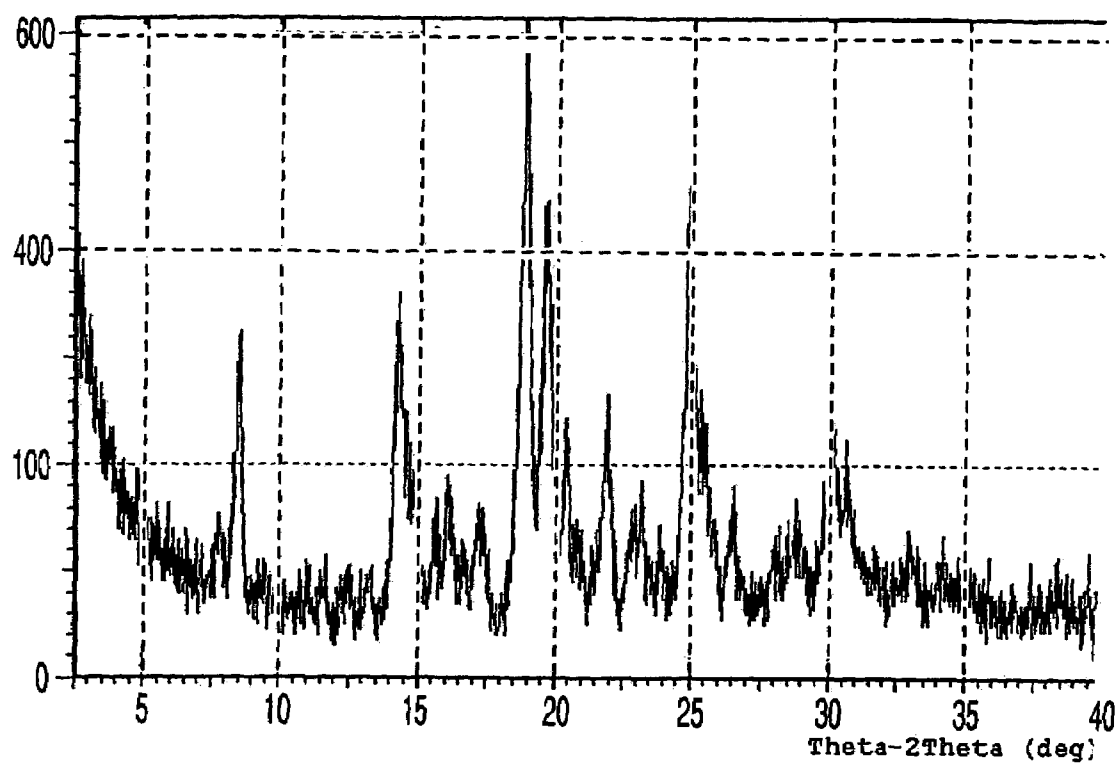
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XRPD PATTERN OF FORM F



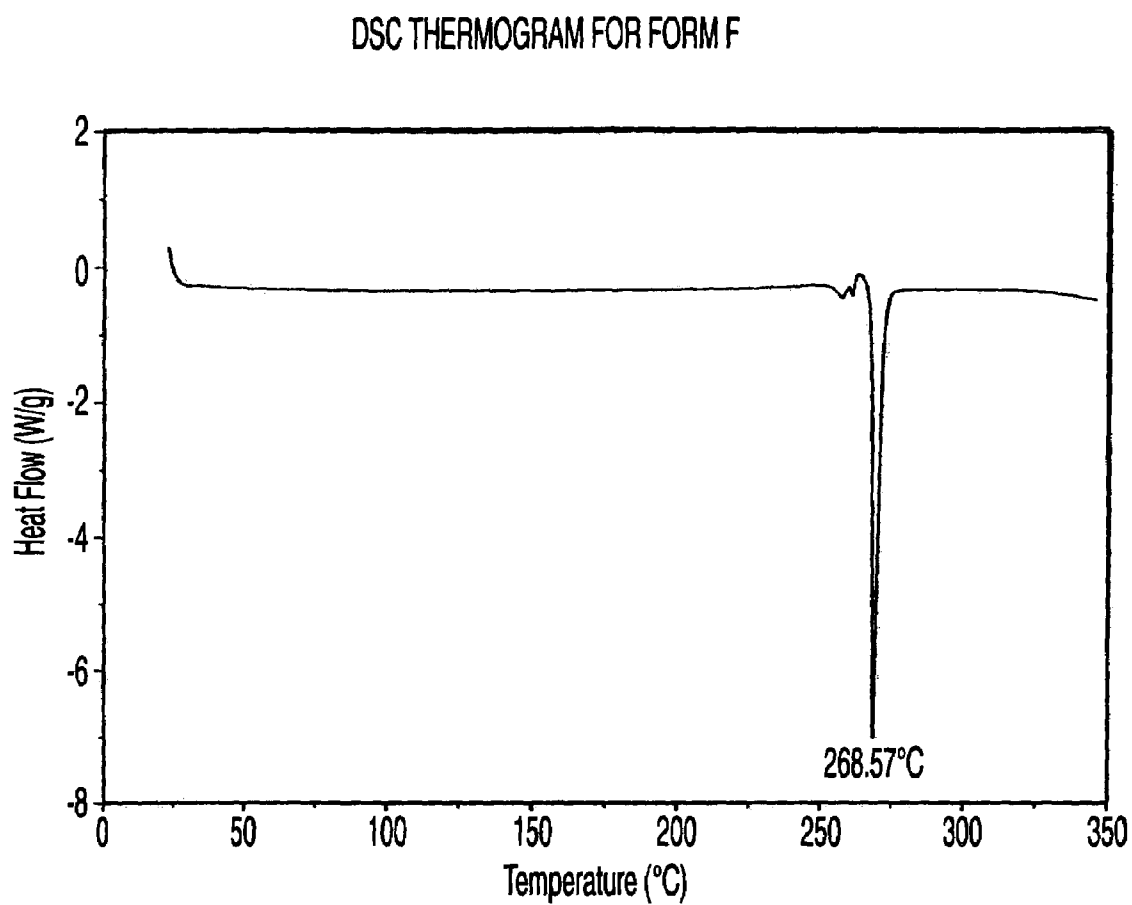
*Fig. 26*

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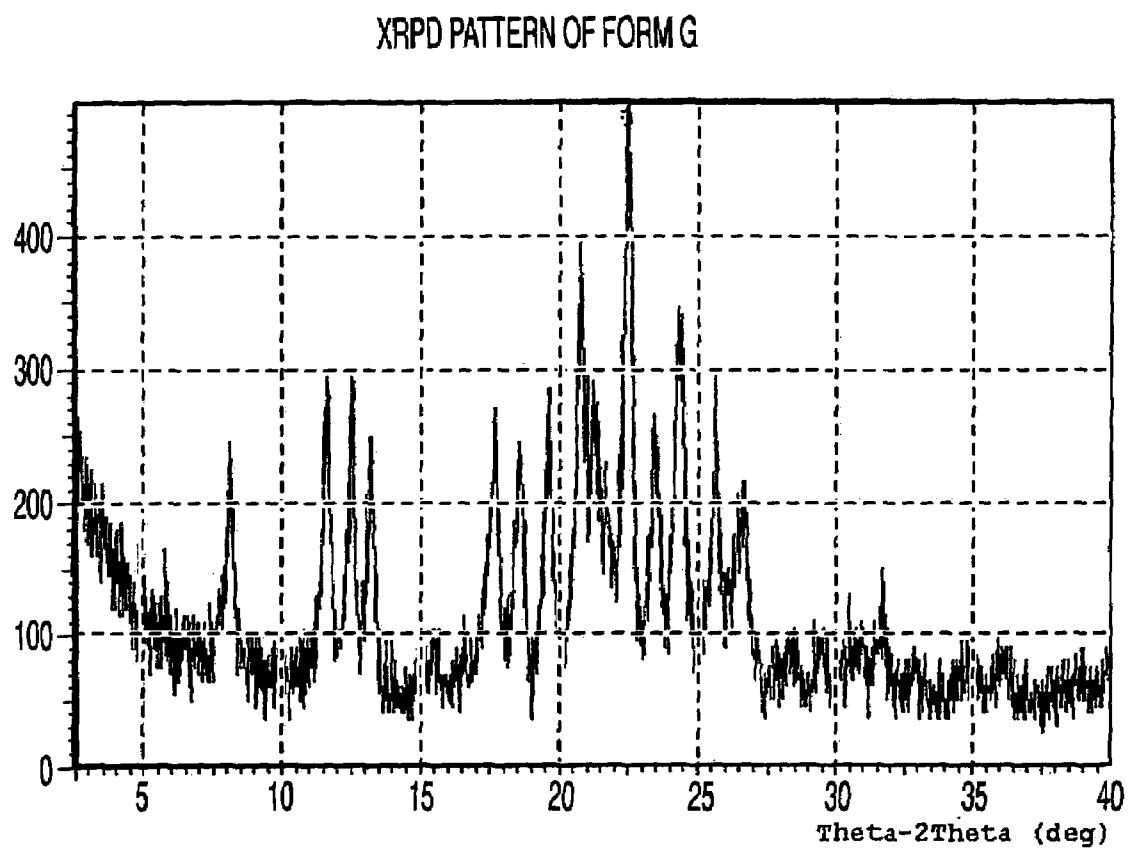
*Fig. 27*

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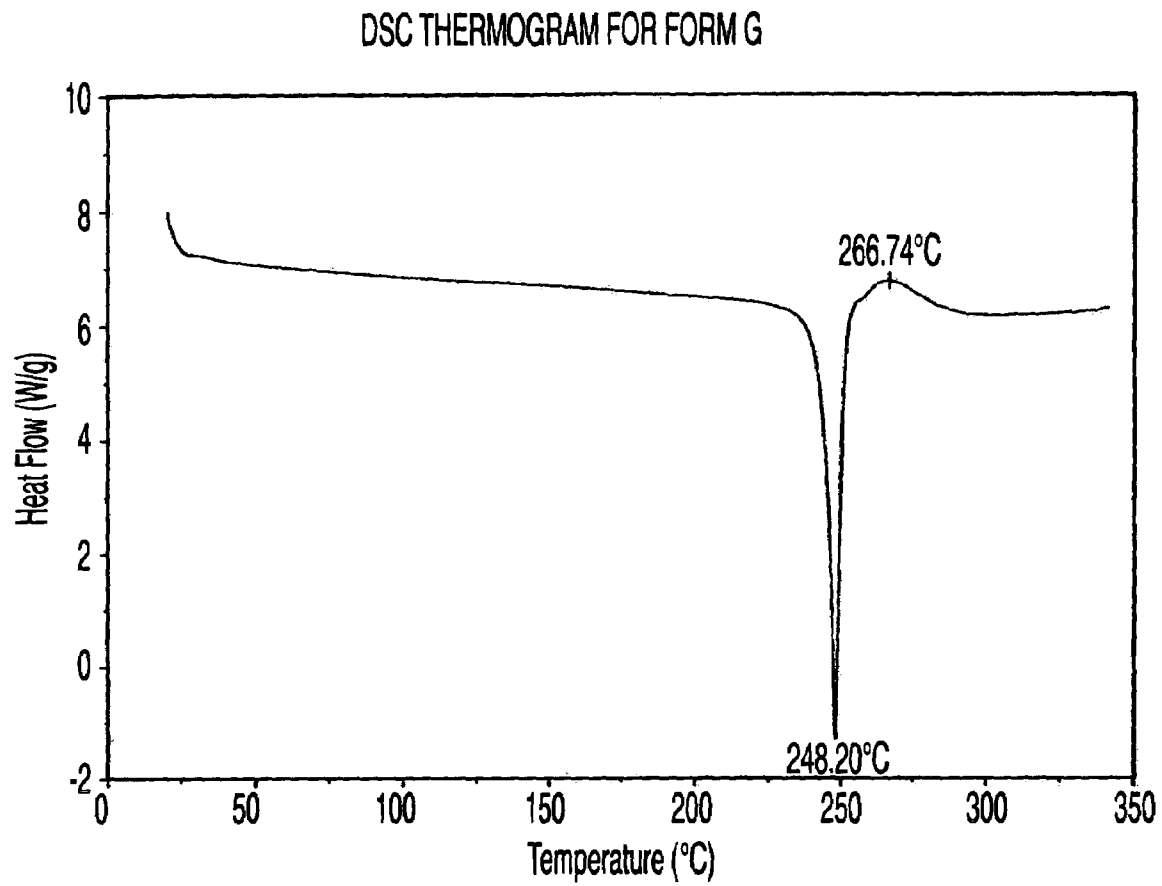
*Fig. 28*

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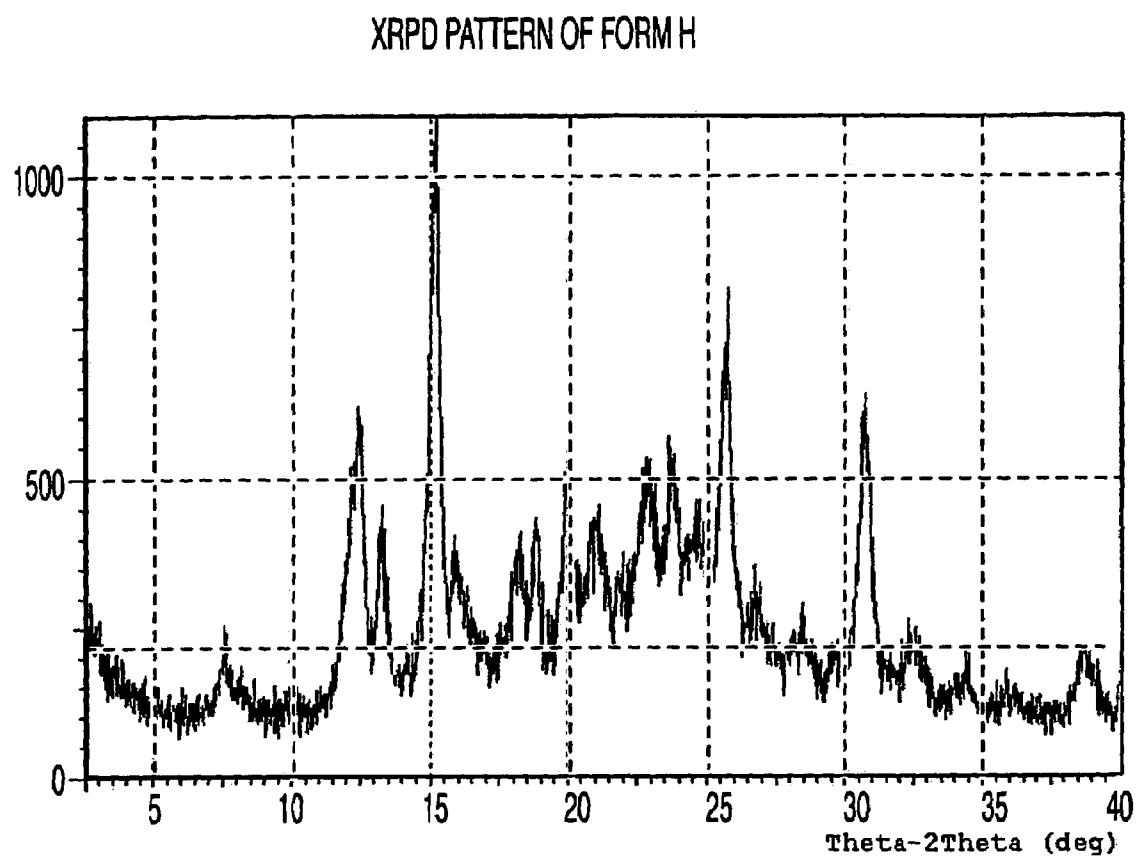
*Fig. 29*

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*Fig. 30*



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## TGA (TOP) AND DSC (BOTTOM) OF FORM H

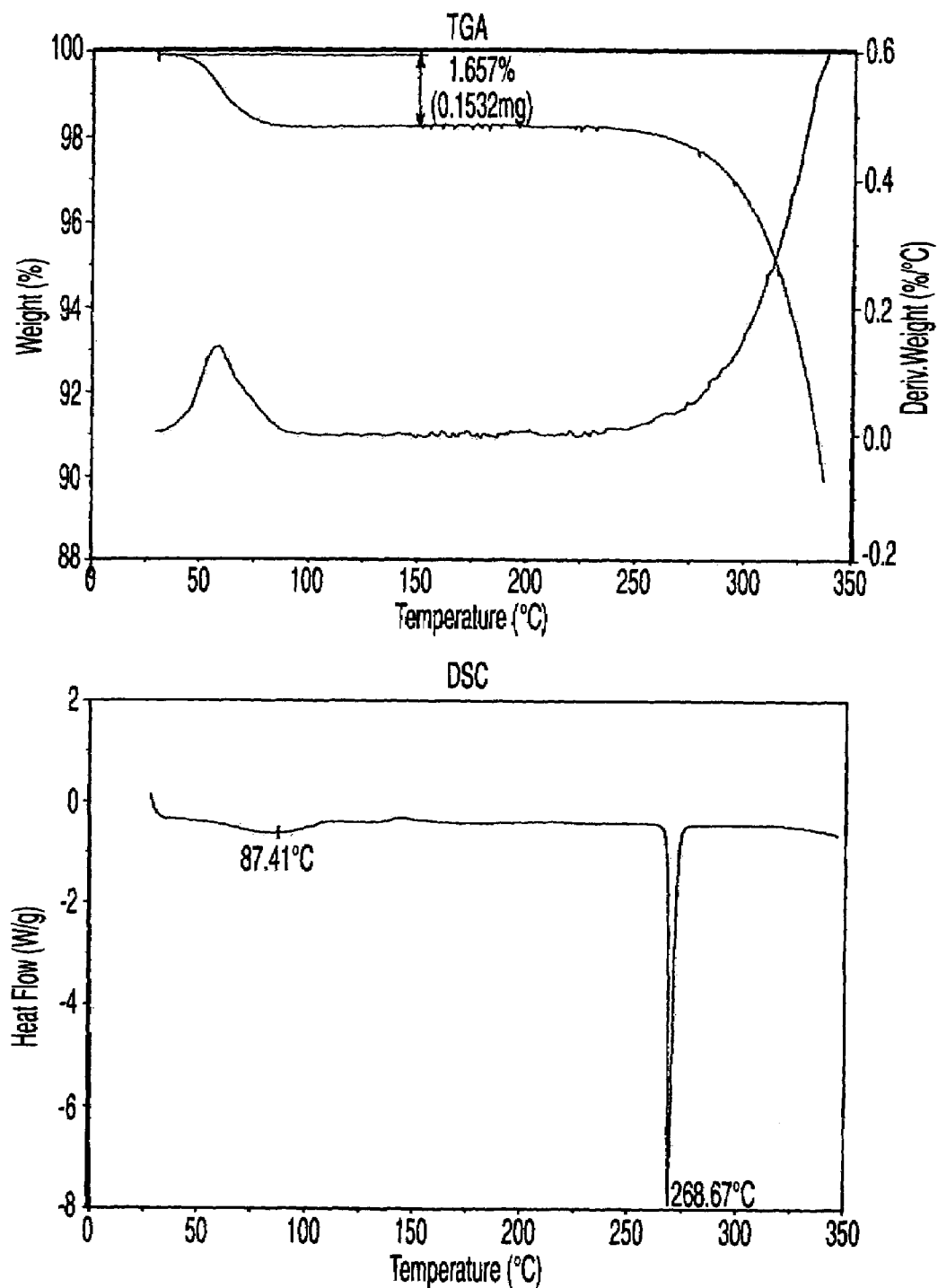


Fig. 31

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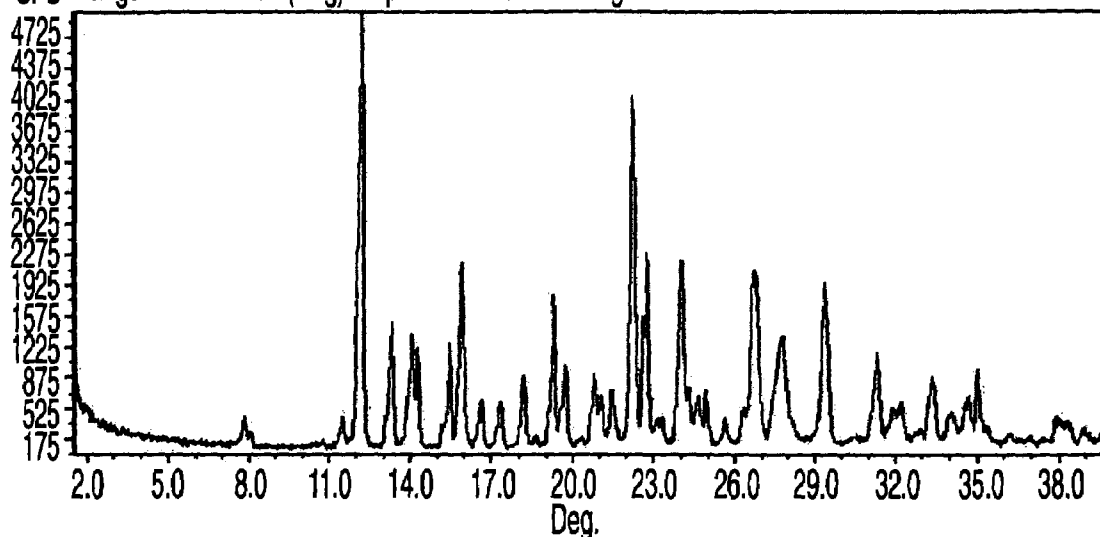
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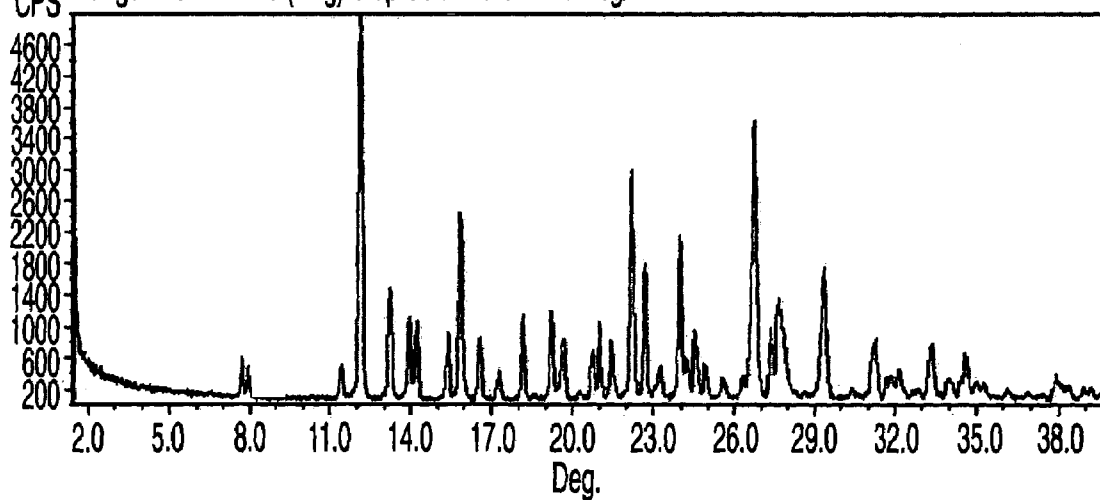
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## XRPD PATTERN OF POLYMORPH B

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CPS Range: 1.50 - 40.00 (Deg) Step Scan Rate: 2.40 Deg/min

*Fig. 32*

File: Process 5274-100-C XRPD PATTERN OF POLYMORPH B  
Date: 06-04-04 16:11 Step: 0.020° Cnt Time: 0.500 Sec.  
CPS Range: 1.50 - 40.00 (Deg) Step Scan Rate: 2.40 Deg/min

*Fig. 33*

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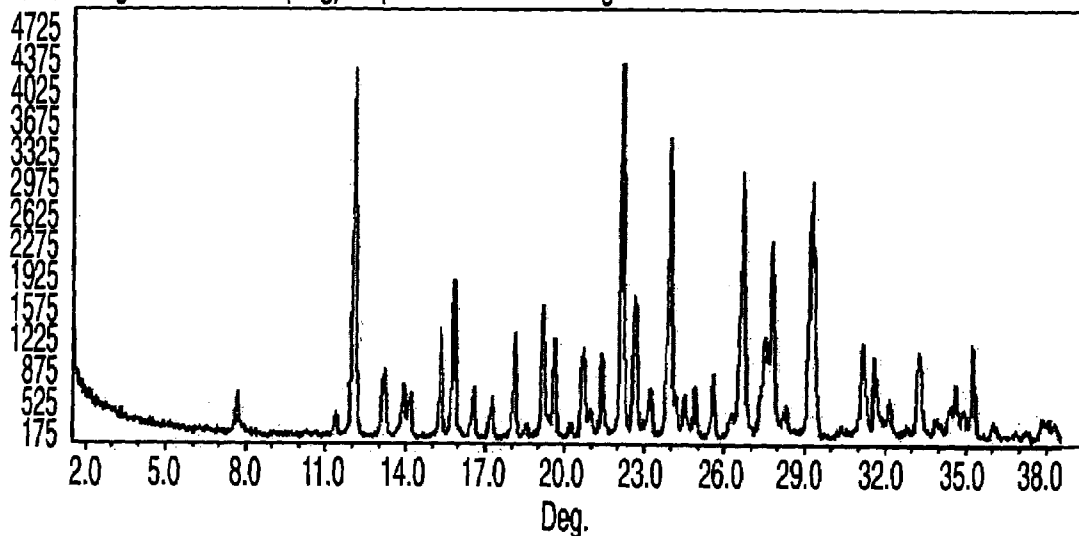
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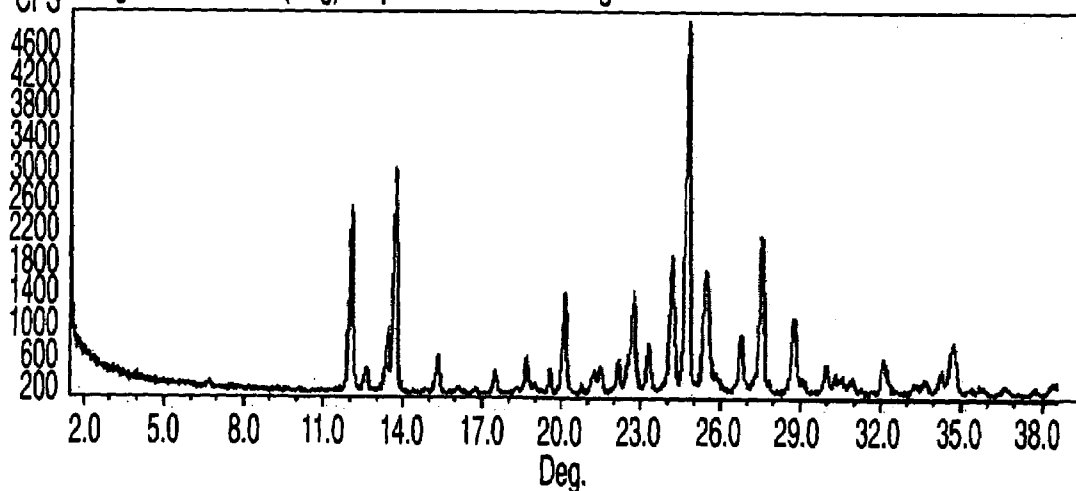
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*Fig. 34*

## XRPD PATTERN OF POLYMORPH E

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*Fig. 35*

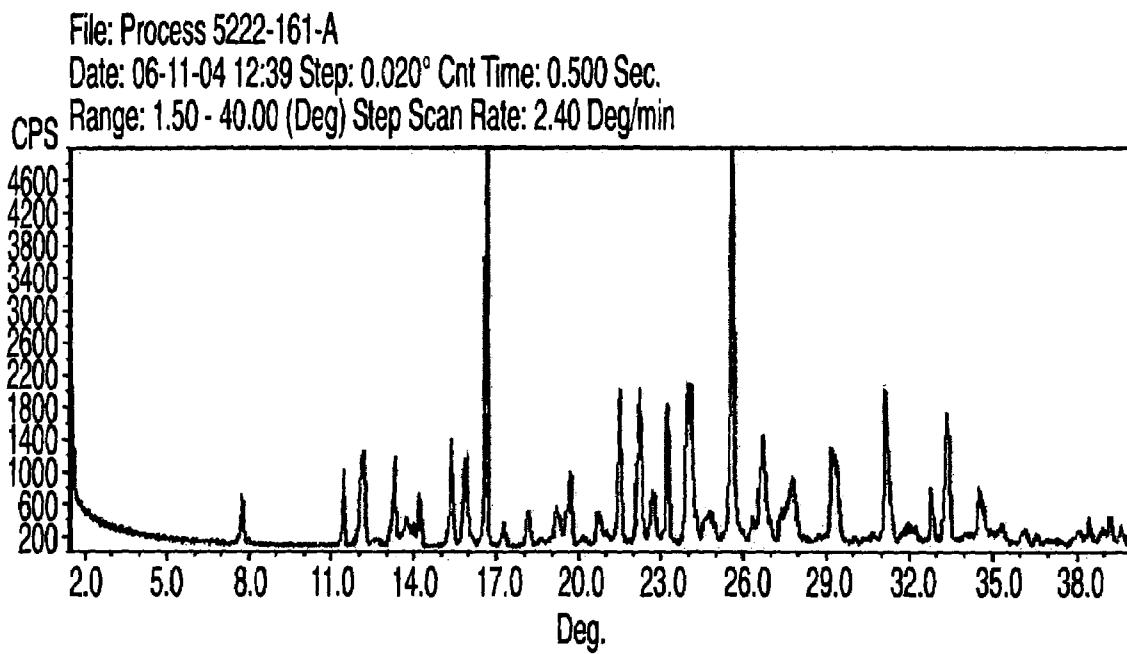
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**XRPD PATTERN OF POLYMORPH MIXTURE**



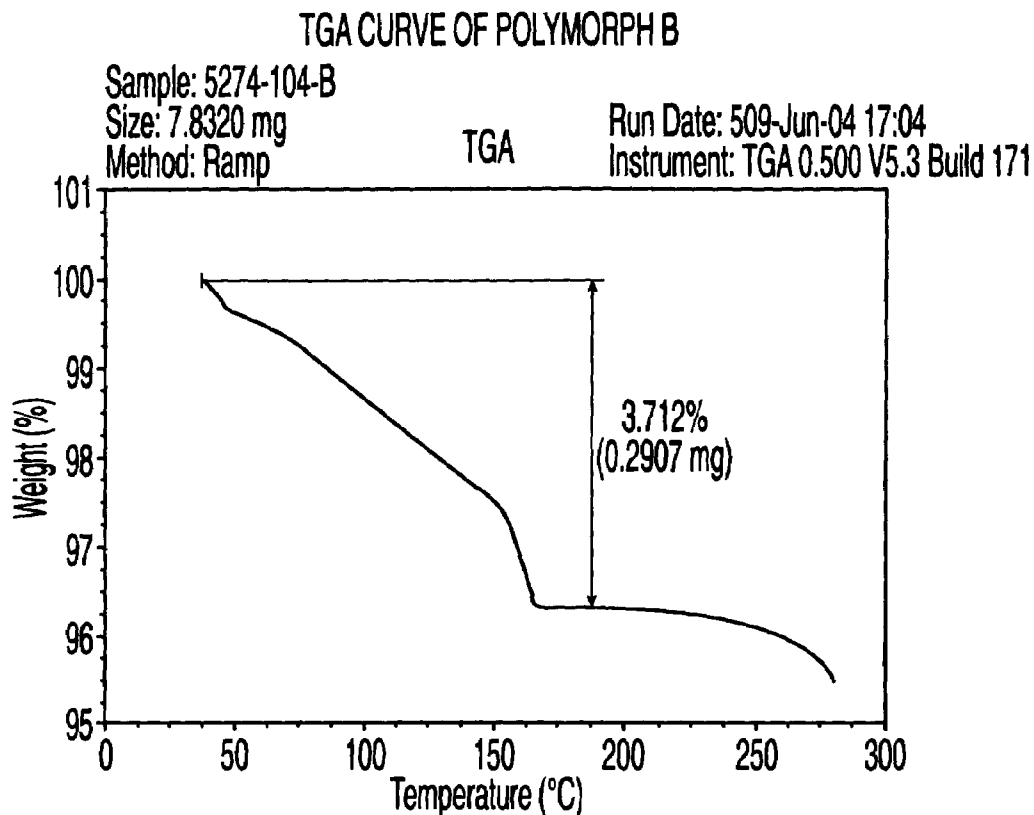
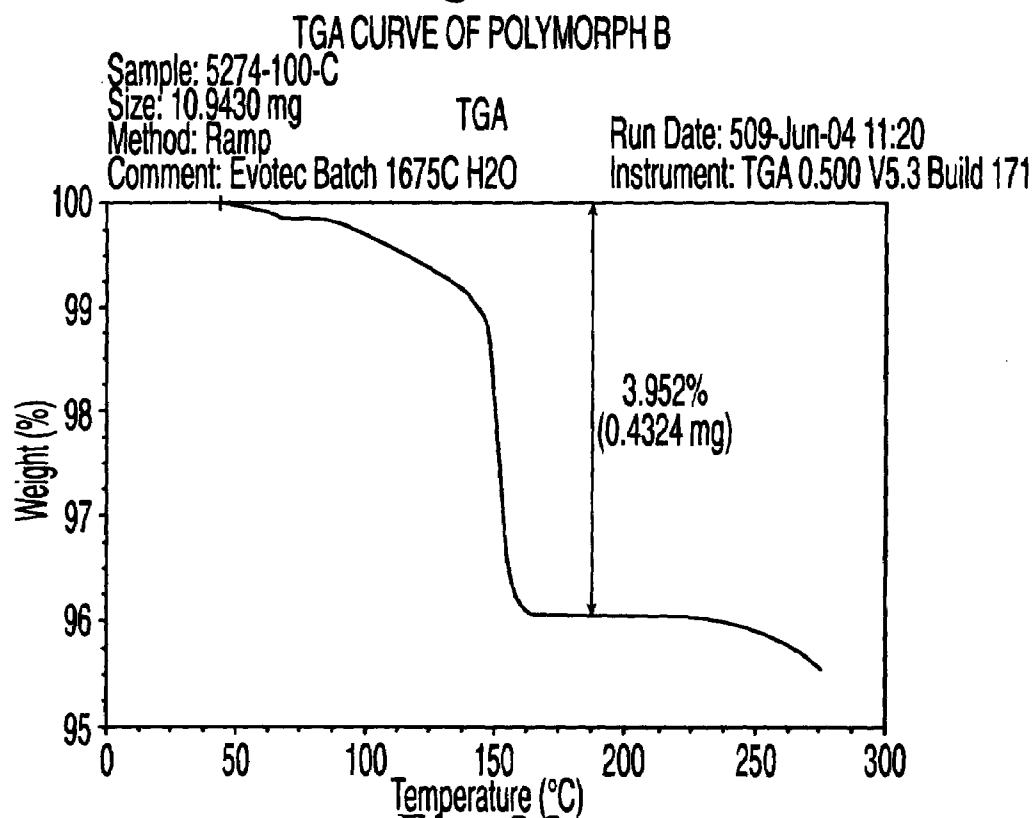
*Fig. 36*

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*Fig. 37**Fig. 38*

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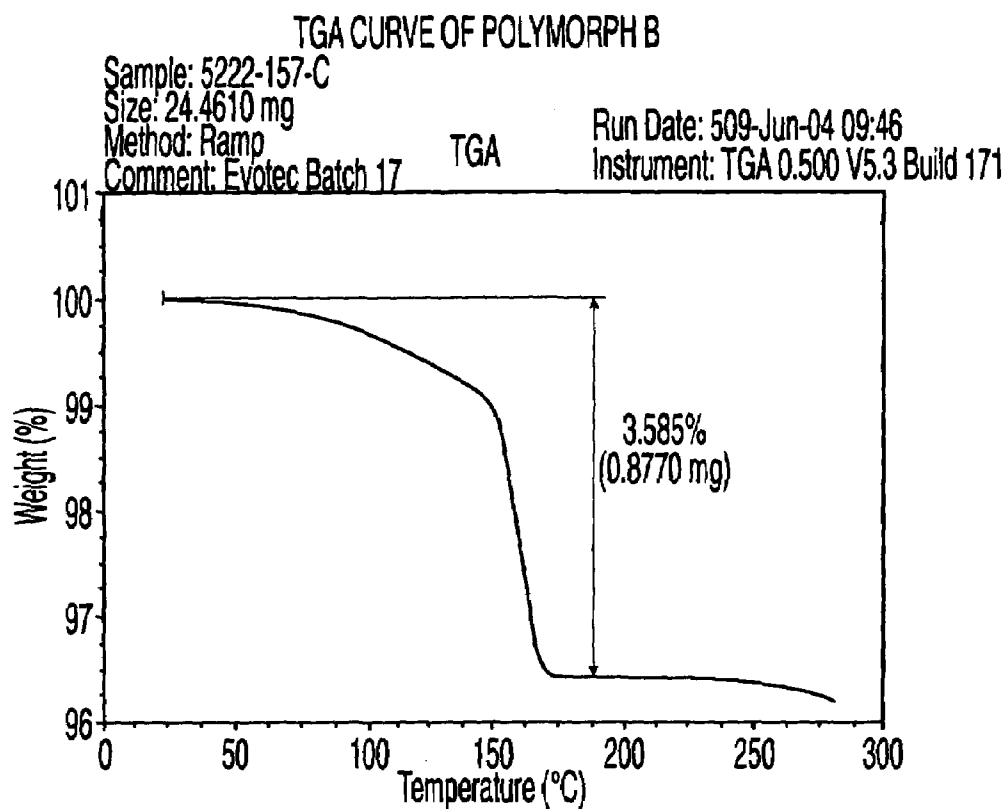


Fig. 39

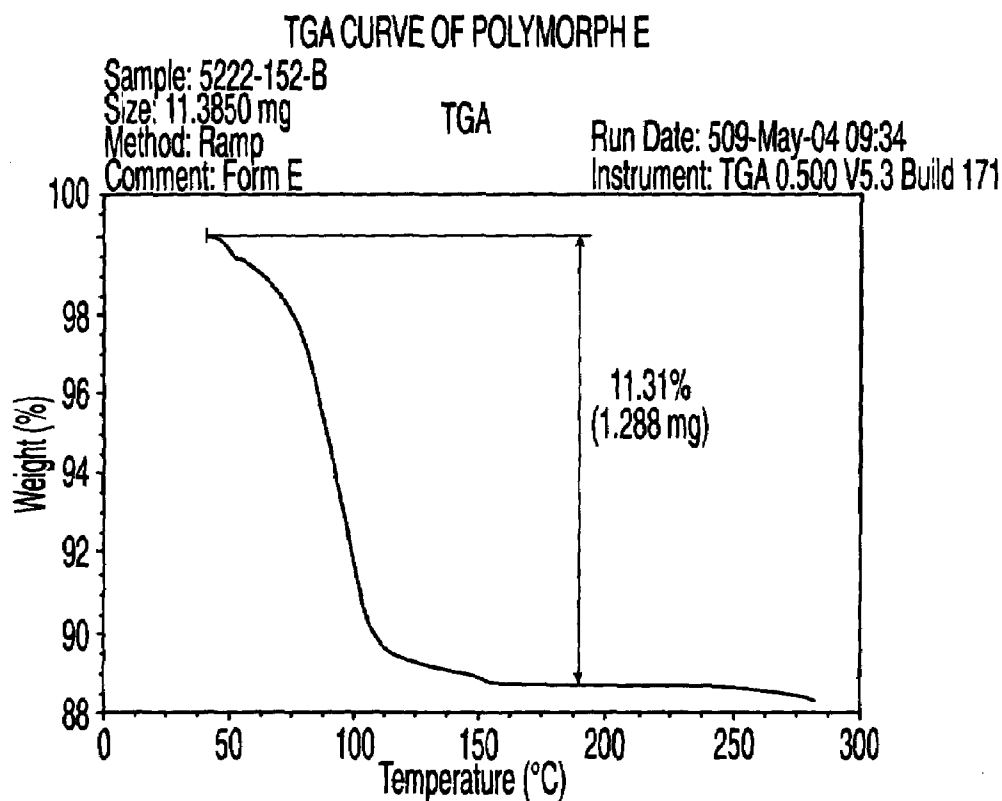


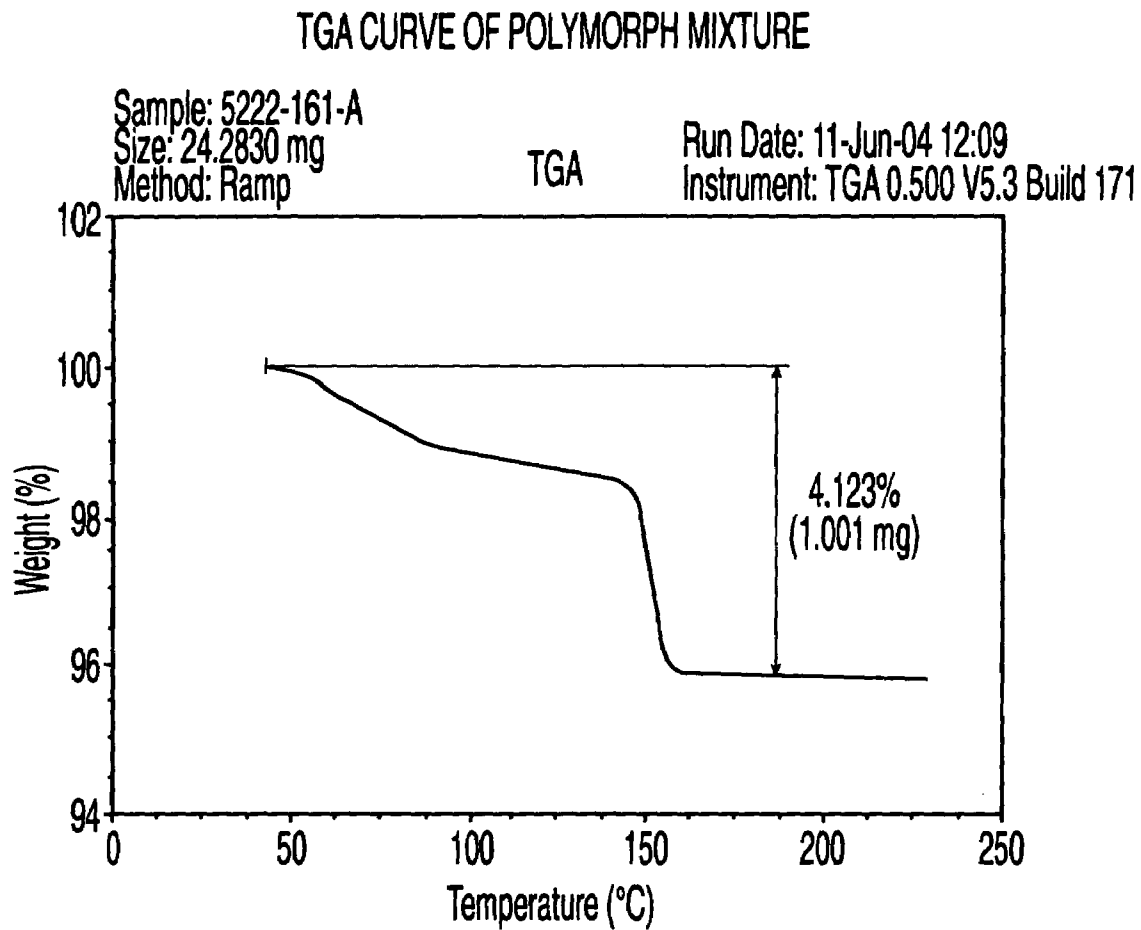
Fig. 40

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*Fig. 41*



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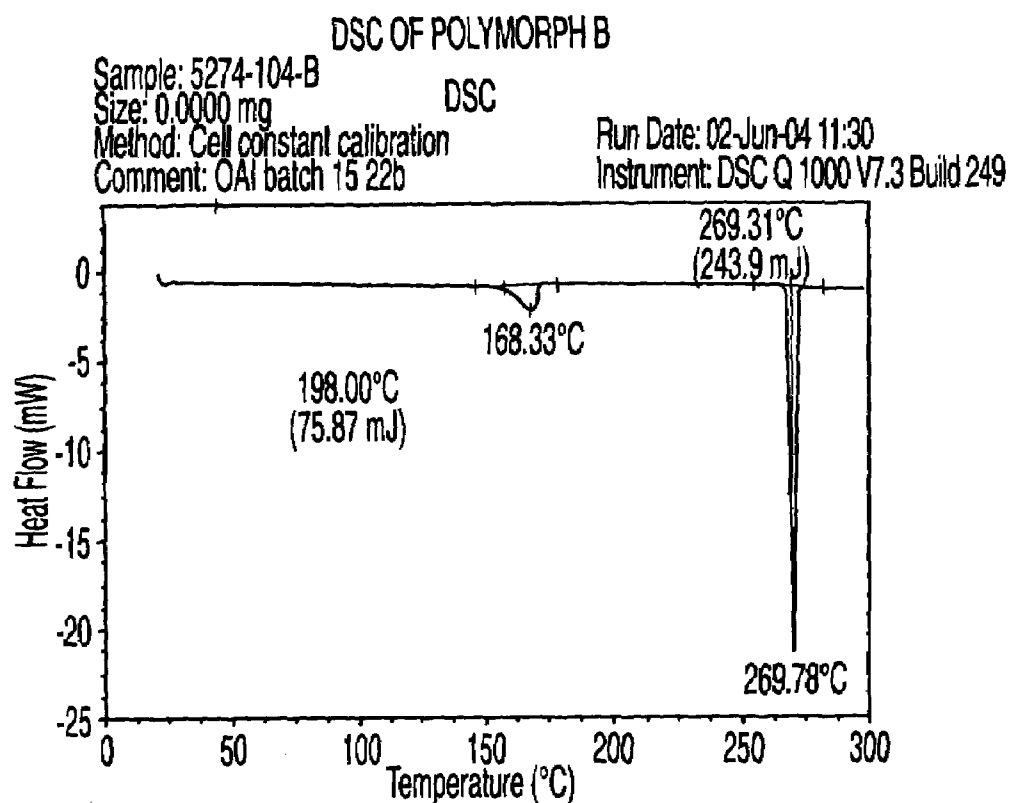


Fig. 42

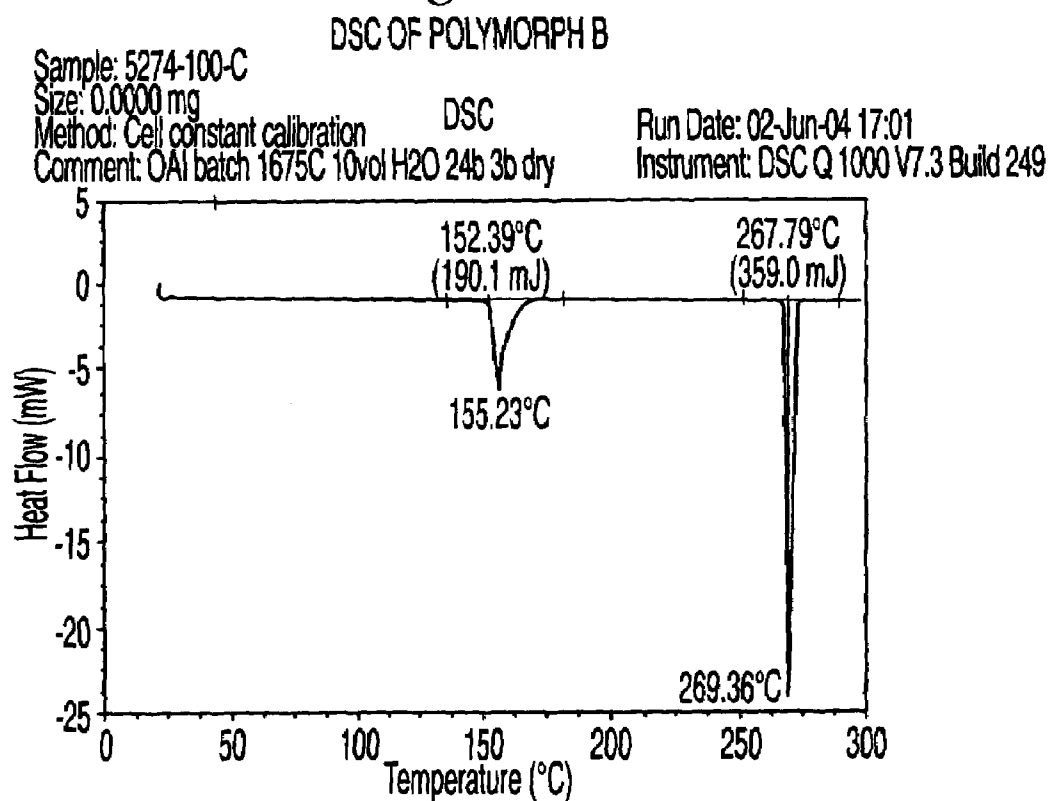


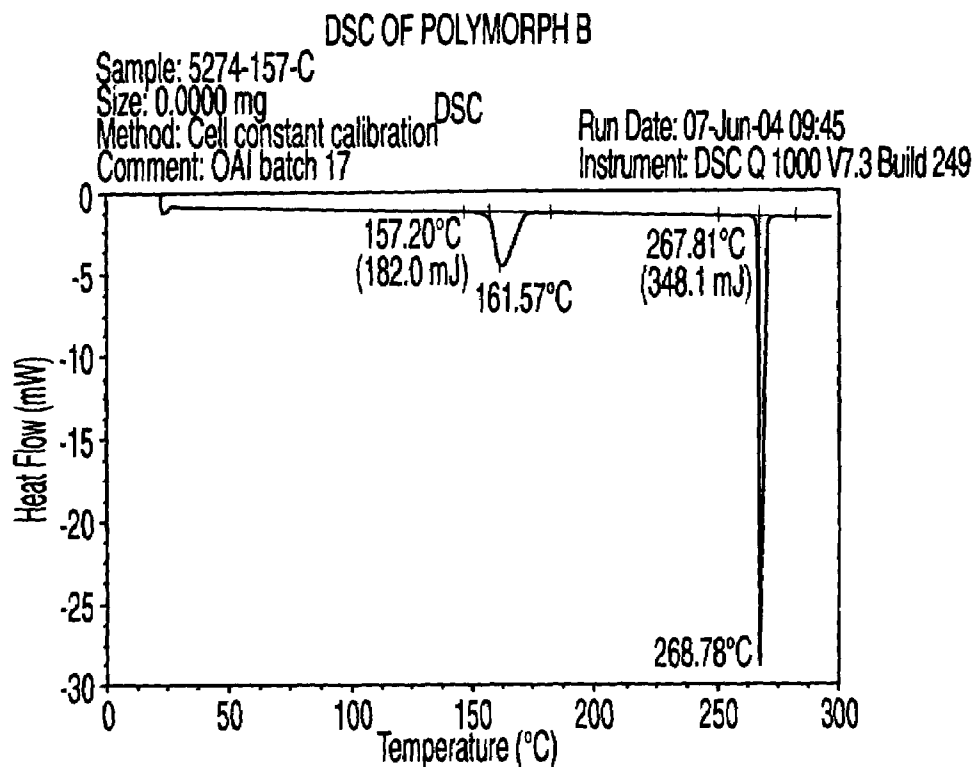
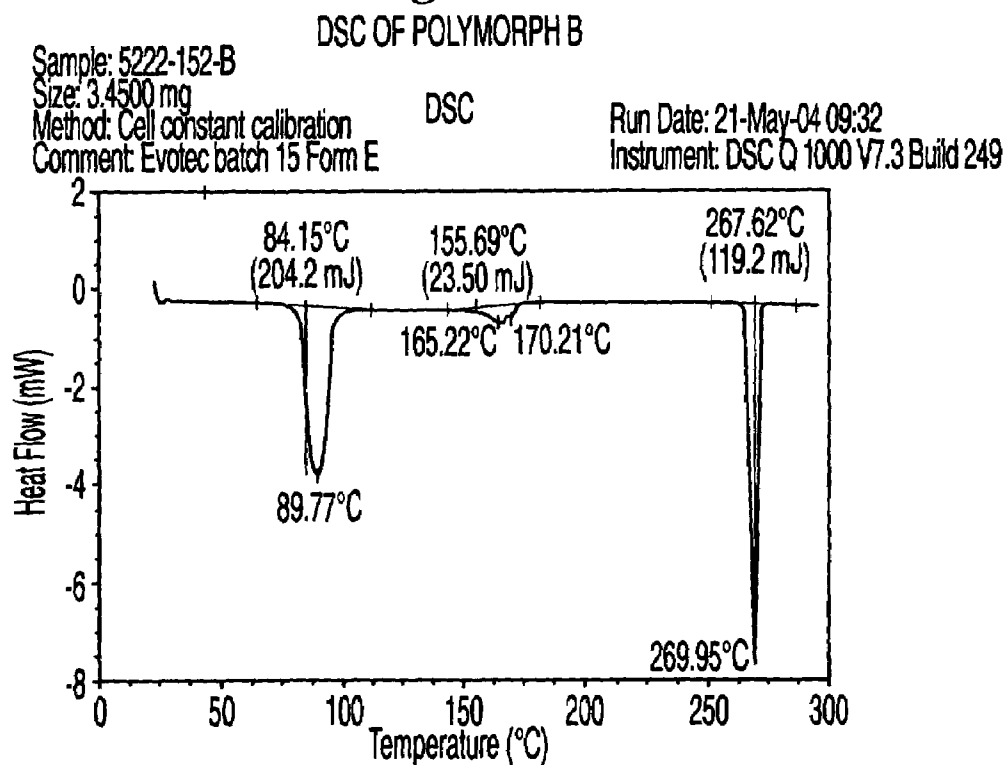
Fig. 43

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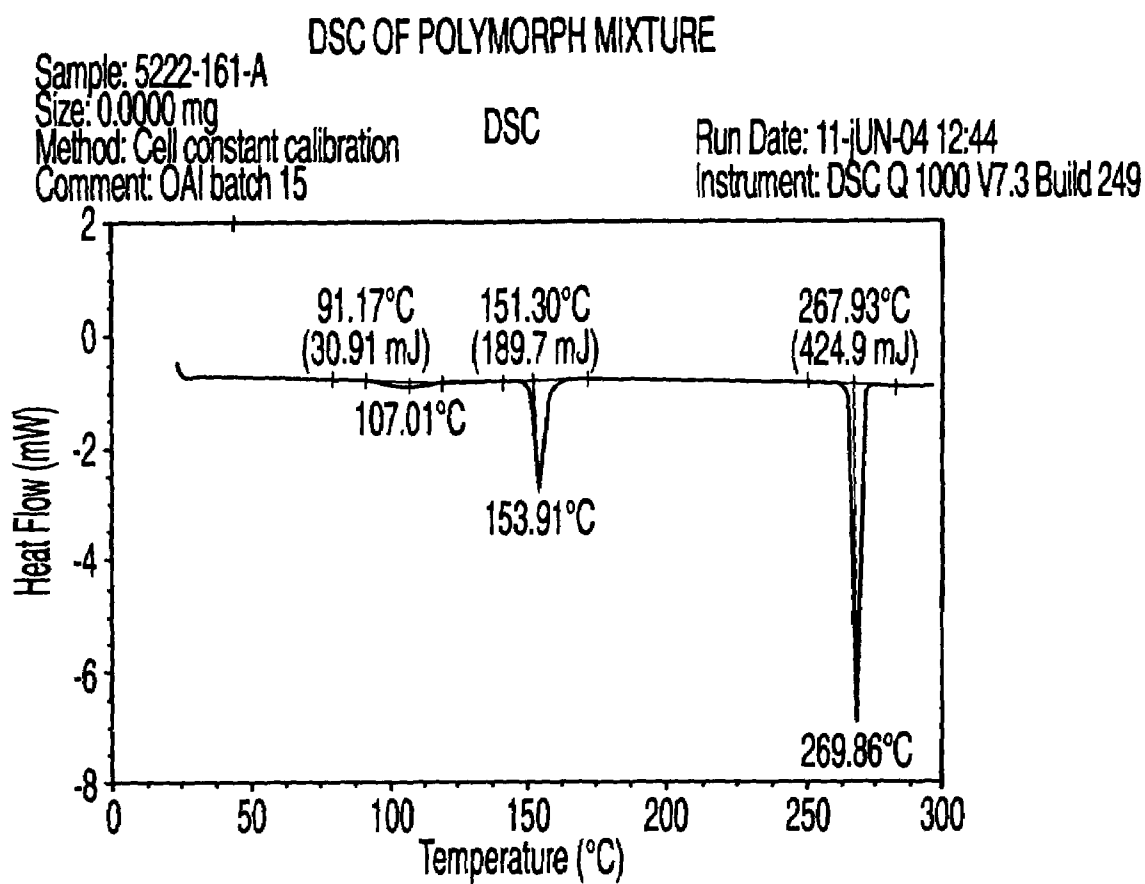
*Fig. 44**Fig. 45*

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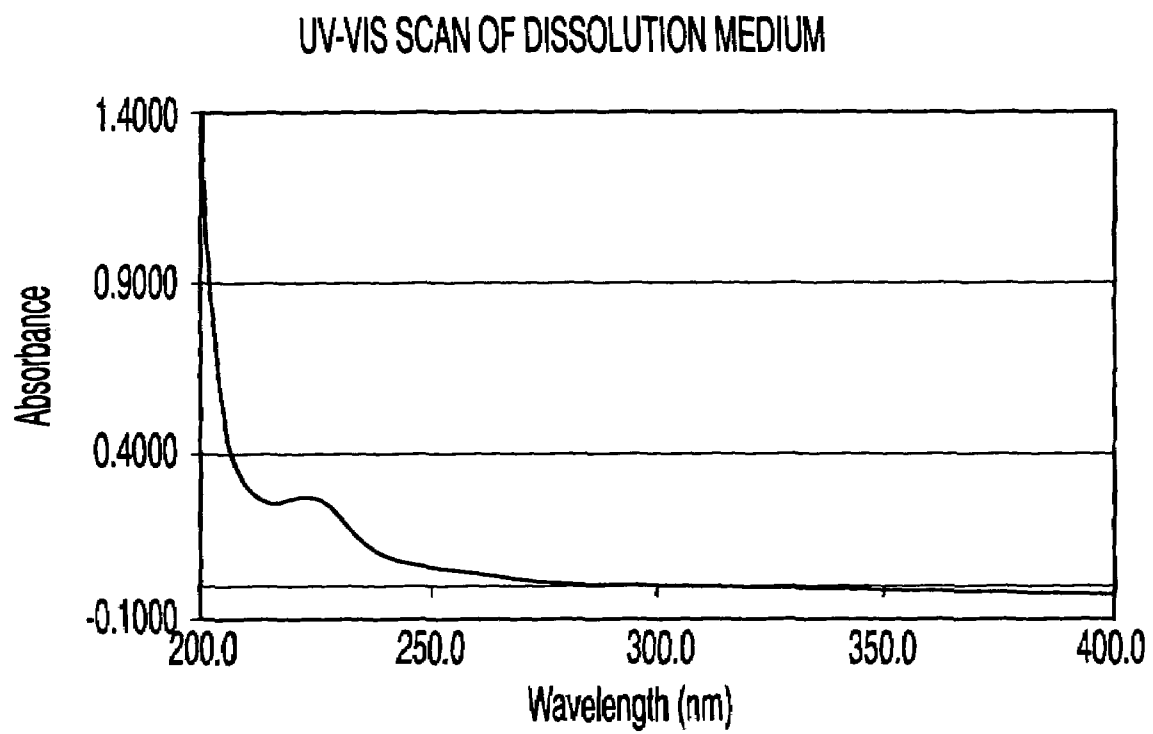
*Fig. 46*

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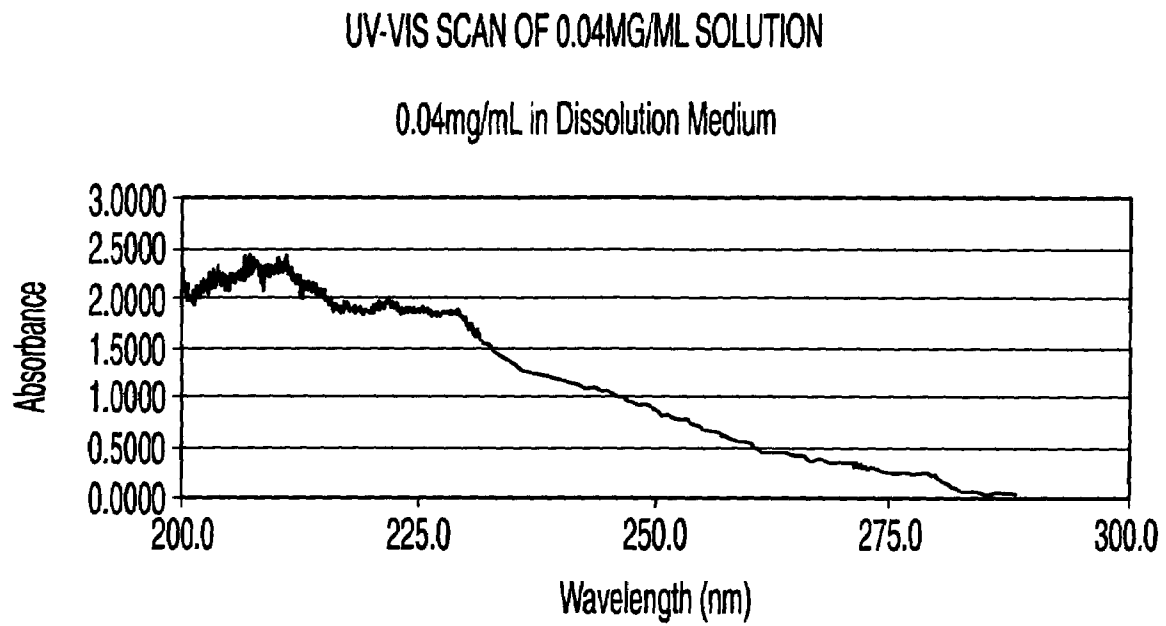
*Fig. 47*

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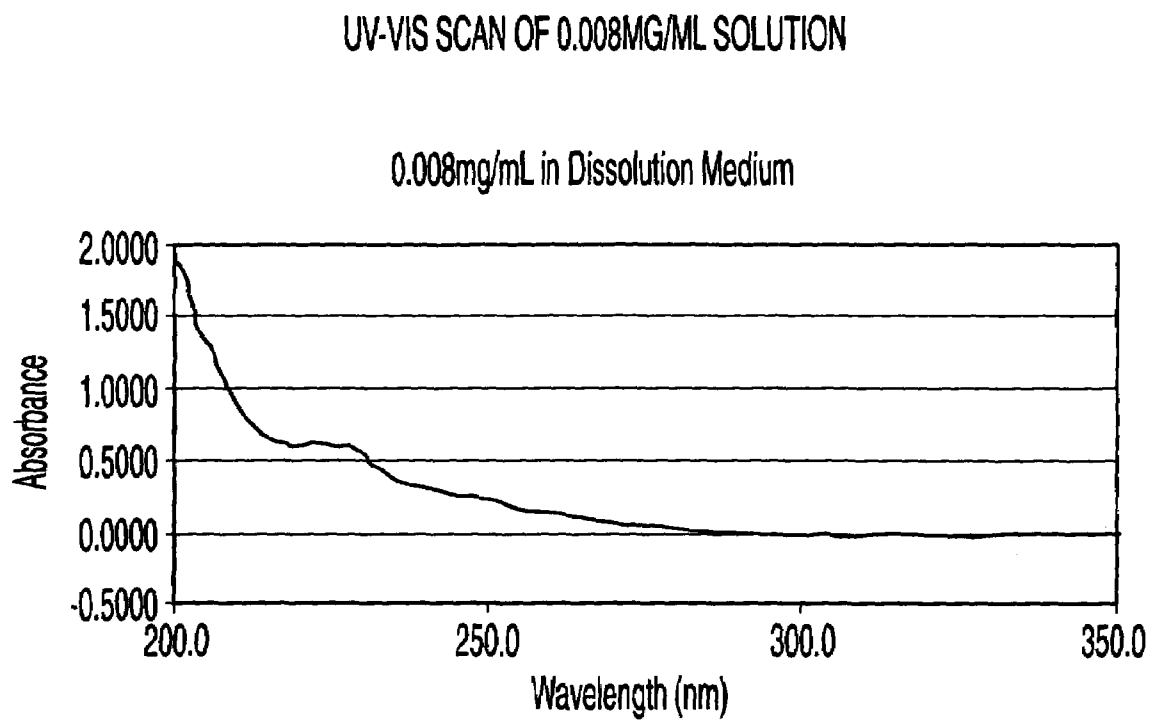
*Fig. 48*

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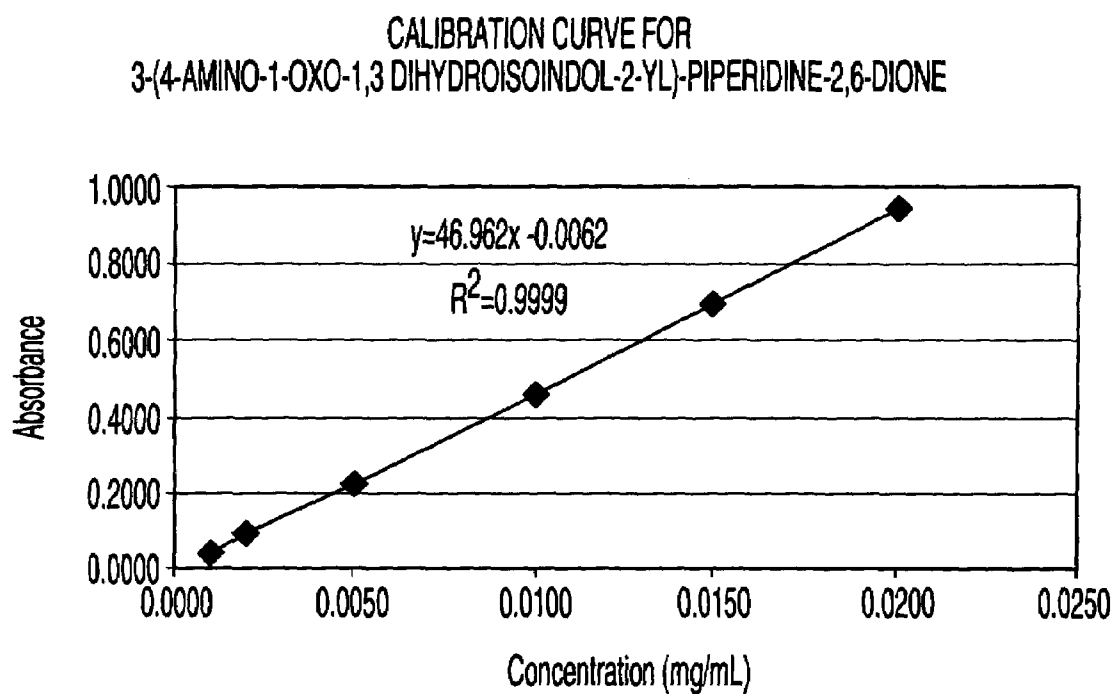
*Fig. 49*

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*Fig. 50*



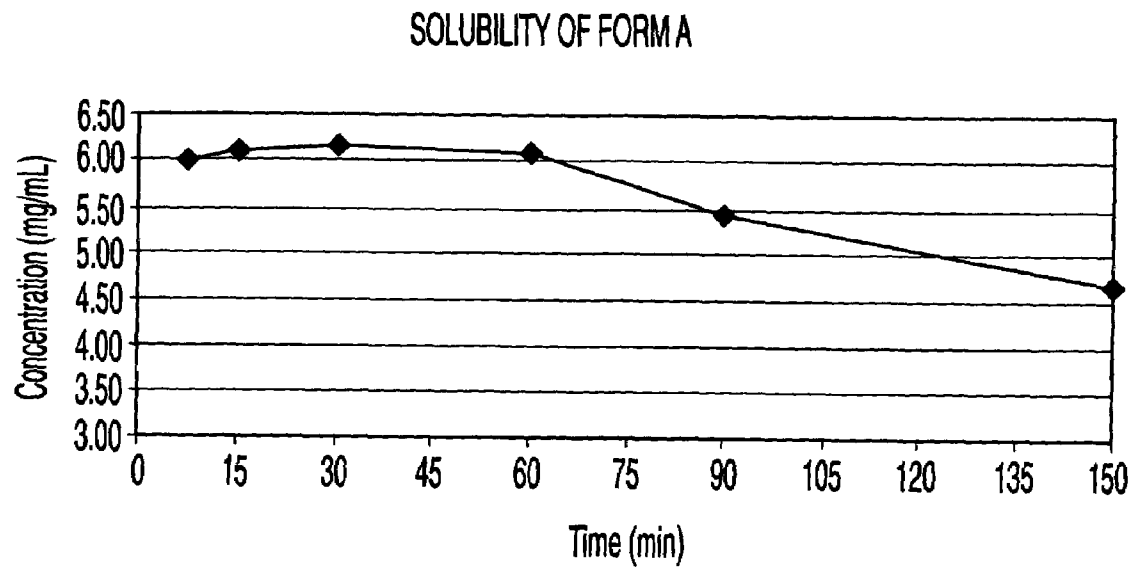


Fig. 51

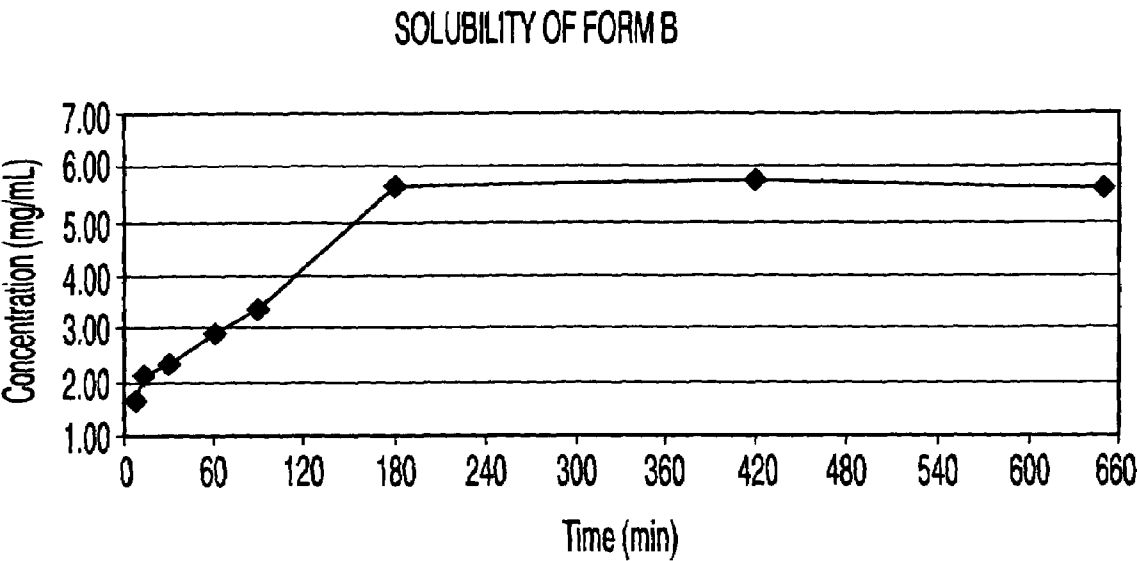


Fig. 52

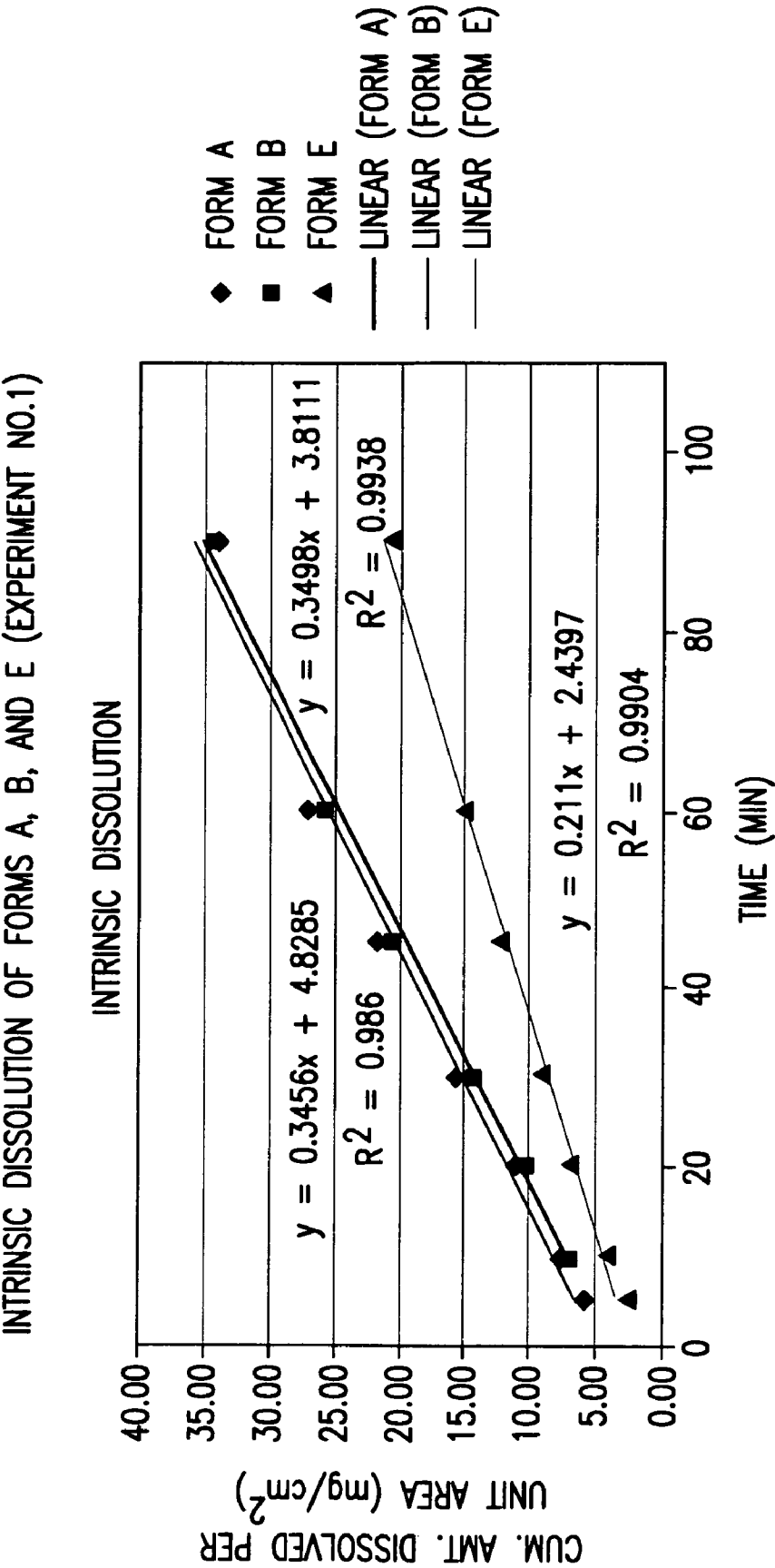


FIG.53

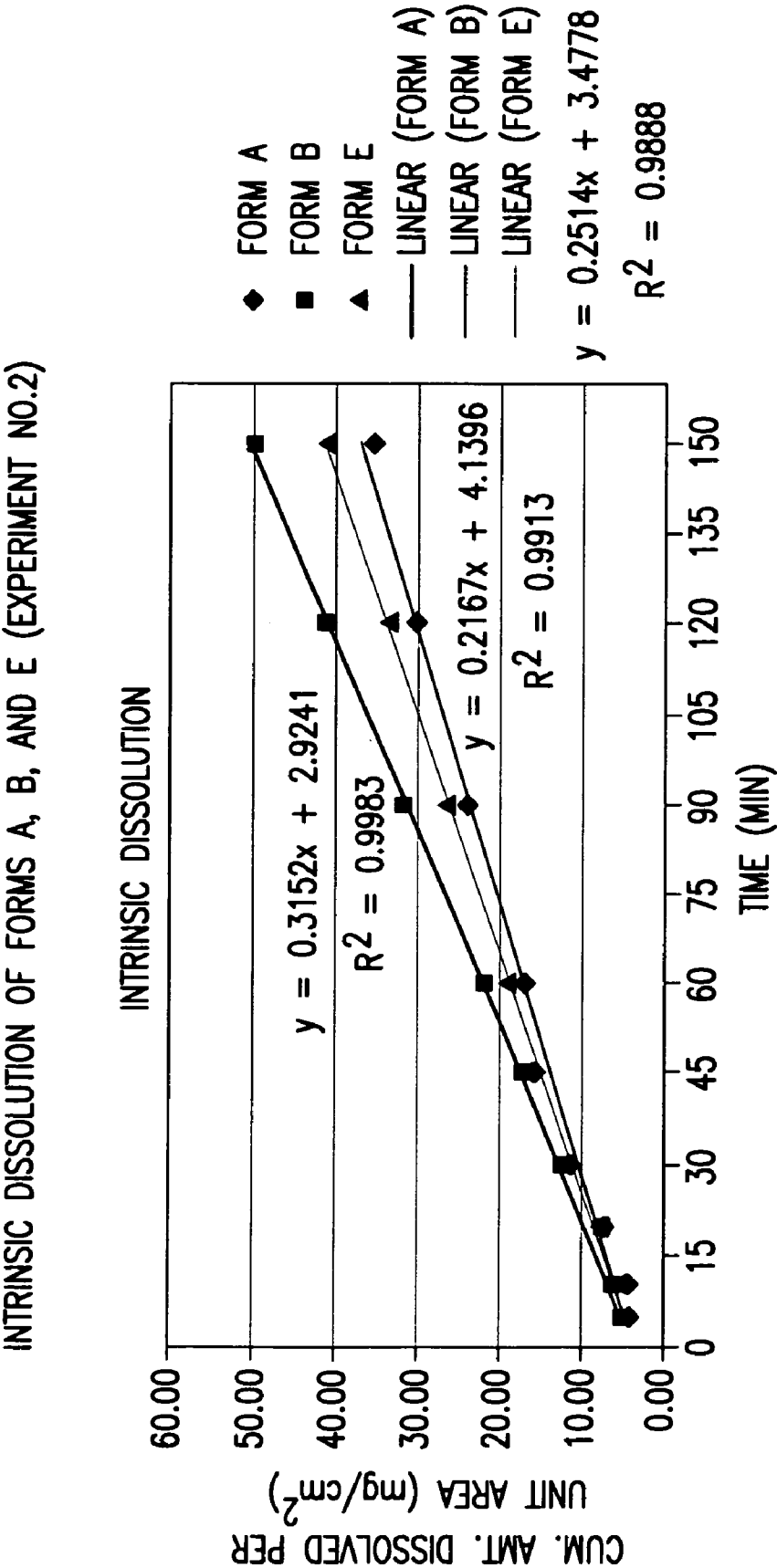


FIG.54

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**POLYMORPHIC FORMS OF  
3-(4-AMINO-1-OXO-1,3  
DIHYDRO-ISOINDOL-2-YL)-PIPERIDINE-2,6-  
IONE**

This application claims the benefit of U.S. provisional application 60/499,723, filed Sep. 4, 2003, the contents of which are incorporated by reference herein their entirety.

### 1. FIELD OF THE INVENTION

This invention relates to polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, compositions comprising the polymorphic forms, methods of making the polymorphic forms and methods of their use for the treatment of diseases and conditions including, but not limited to, inflammatory diseases, autoimmune diseases, and cancer.

### 2. BACKGROUND OF THE INVENTION

Many compounds can exist in different crystal forms, or polymorphs, which exhibit different physical, chemical, and spectroscopic properties. For example, certain polymorphs of a compound may be more readily soluble in particular solvents, may flow more readily, or may compress more easily than others. See, e.g., P. DiMartino, et al., *J. Thermal Anal.*, 48:447-458 (1997). In the case of drugs, certain solid forms may be more bioavailable than others, while others may be more stable under certain manufacturing, storage, and biological conditions. This is particularly important from a regulatory standpoint, since drugs are approved by agencies such as the U.S. Food and Drug Administration only if they meet exacting purity and characterization standards. Indeed, the regulatory approval of one polymorph of a compound, which exhibits certain solubility and physico-chemical (including spectroscopic) properties, typically does not imply the ready approval of other polymorphs of that same compound.

Polymorphic forms of a compound are known in the pharmaceutical arts to affect, for example, the solubility, stability, flowability, fractability, and compressibility of the compound, as well as the safety and efficacy of drug products comprising it. See, e.g., Knapman, K. *Modern Drug Discoveries*, 2000, 53. Therefore, the discovery of new polymorphs of a drug can provide a variety of advantages.

U.S. Pat. Nos. 5,635,517 and 6,281,230, both to Muller et al., disclose 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, which is useful in treating and preventing a wide range of diseases and conditions including, but not limited to, inflammatory diseases, autoimmune diseases, and cancer. New polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can further the development of formulations for the treatment of these chronic illnesses, and may yield numerous formulation, manufacturing and therapeutic benefits.

### 3. SUMMARY OF THE INVENTION

This invention encompasses polymorphs of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. In certain aspects, the invention provides polymorphs of the compound identified herein as forms A, B, C, D, E, F, G, and H. The invention also encompasses mixtures of these forms. In further embodiments, this invention provides methods of making, isolating and characterizing the polymorphs.

This invention also provides pharmaceutical compositions and single unit dosage forms comprising a polymorph of

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3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The invention further provides methods for the treatment or prevention of a variety of diseases and disorders, which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of a polymorph of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

### 4. BRIEF DESCRIPTION OF THE DRAWINGS

Specific aspects of the invention can be understood with reference to the attached figures:

FIG. 1 provides a representative X-ray powder diffraction (XRPD) pattern of Form A;

FIG. 2 provides a representative IR spectrum of Form A;

FIG. 3 provides a representative Raman spectrum of Form A;

FIG. 4 provides a representative thermogravimetric analysis (TGA) curve and a representative differential scanning calorimeter (DSC) thermogram of Form A;

FIG. 5 provides a representative moisture sorption/desorption isotherm of Form A;

FIG. 6 provides a representative XRPD pattern of Form B;

FIG. 7 provides a representative IR spectrum of Form B;

FIG. 8 provides a representative Raman spectrum of Form B;

FIG. 9 provides a representative TGA curve and a representative DSC thermogram of Form B;

FIG. 10 provides representative TG-IR results of Form B;

FIG. 11 provides a representative moisture sorption/desorption isotherm of Form B;

FIG. 12 provides a representative XRPD pattern of Form C;

FIG. 13 provides a representative IR spectrum of Form C;

FIG. 14 provides a representative Raman spectrum of Form C;

FIG. 15 provides a representative TGA curve and a representative DSC thermogram of Form C;

FIG. 16 provides representative TG-IR results of Form C;

FIG. 17 provides a representative moisture sorption/desorption isotherm of Form C;

FIG. 18 provides a representative XRPD pattern of Form D;

FIG. 19 provides a representative IR spectrum of Form D;

FIG. 20 provides a representative Raman spectrum of Form D;

FIG. 21 provides a representative TGA curve and a representative DSC thermogram of Form D;

FIG. 22 provides a representative moisture sorption/desorption isotherm of Form D;

FIG. 23 provides a representative XRPD pattern of Form E;

FIG. 24 provides a representative TGA curve and a representative DSC thermogram of Form E;

FIG. 25 provides a representative moisture sorption/desorption isotherm of Form E;

FIG. 26 provides a representative XRPD pattern for a sample of Form F;

FIG. 27 provides a representative thermogram of Form F;

FIG. 28 provides a representative XRPD pattern of Form G;

FIG. 29 provides a representative DSC thermogram for a sample of Form G;

FIG. 30 provides a representative XRPD pattern of Form H;

FIG. 31 provides a representative TGA curve and a representative DSC thermogram of Form H;

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FIG. 32 provides a representative XRPD pattern of Form B;

FIG. 33 provides a representative XRPD pattern of Form B;

FIG. 34 provides a representative XRPD pattern of Form B;

FIG. 35 provides a representative XRPD pattern of Form E;

FIG. 36 provides a representative XRPD pattern of polymorph mixture;

FIG. 37 provides a representative TGA curve of Form B;

FIG. 38 provides a representative TGA curve of Form B;

FIG. 39 provides a representative TGA curve of Form B;

FIG. 40 provides a representative TGA curve of Form E;

FIG. 41 provides a representative TGA curve of polymorph mixture;

FIG. 42 provides a representative DSC thermogram of Form B;

FIG. 43 provides a representative DSC thermogram of Form B;

FIG. 44 provides a representative DSC thermogram of Form B;

FIG. 45 provides a representative DSC thermogram of Form E;

FIG. 46 provides a representative DSC thermogram of polymorph mixture;

FIG. 47 provides a UV-Vis scan of dissolution medium;

FIG. 48 provides a UV-Vis scan of 0.04 mg/ml of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in dissolution medium;

FIG. 49 provides a UV-Vis scan of 0.008 mg/ml of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in dissolution medium;

FIG. 50 provides a calibration curve for 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione;

FIG. 51 provides a solubility curve of Form A;

FIG. 52 provides a solubility curve of Form B;

FIG. 53 provides an intrinsic dissolution of Forms A, B and E; and

FIG. 54 provides an intrinsic dissolution of Forms A, B and E.

## 5. DETAILED DESCRIPTION OF THE INVENTION

### 5.1 Definitions

As used herein and unless otherwise indicated, the terms “treat,” “treating” and “treatment” refer to the alleviation of a disease or disorder and/or at least one of its attendant symptoms.

As used herein and unless otherwise indicated, the terms “prevent,” “preventing” and “prevention” refer to the inhibition of a symptom of a disease or disorder or the disease itself.

As used herein and unless otherwise indicated, the terms “polymorph” and “polymorphic form” refer to solid crystalline forms of a compound or complex. Different polymorphs of the same compound can exhibit different physical, chemical and/or spectroscopic properties. Different physical properties include, but are not limited to stability (e.g., to heat or light), compressibility and density (important in formulation and product manufacturing), and dissolution rates (which can affect bioavailability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical characteristics (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both

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(e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). Different physical properties of polymorphs can affect their processing. For example, one polymorph might be more likely to form solvates or might be more difficult to filter or wash free of impurities than another due to, for example, the shape or size distribution of particles of it.

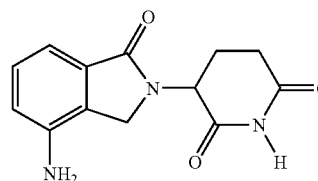
Polymorphs of a molecule can be obtained by a number of methods known in the art. Such methods include, but are not limited to, melt recrystallization, melt cooling, solvent recrystallization, desolvation, rapid evaporation, rapid cooling, slow cooling, vapor diffusion and sublimation. Polymorphs can be detected, identified, classified and characterized using well-known techniques such as, but not limited to, differential scanning calorimetry (DSC), thermogravimetry (TGA), X-ray powder diffractometry (XRPD), single crystal X-ray diffractometry, vibrational spectroscopy, solution calorimetry, solid state nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, Raman spectroscopy, hot stage optical microscopy, scanning electron microscopy (SEM), electron crystallography and quantitative analysis, particle size analysis (PSA), surface area analysis, solubility, and rate of dissolution.

As used herein to refer to the spectra or data presented in graphical form (e.g., XRPD, IR, Raman and NMR spectra), and unless otherwise indicated, the term “peak” refers to a peak or other special feature that one skilled in the art would recognize as not attributable to background noise. The term “significant peaks” refers to peaks at least the median size (e.g., height) of other peaks in the spectrum or data, or at least 1.5, 2, or 2.5 times the median size of other peaks in the spectrum or data.

As used herein and unless otherwise indicated, the term “substantially pure” when used to describe a polymorph of a compound means a solid form of the compound that comprises that polymorph and is substantially free of other polymorphs of the compound. A representative substantially pure polymorph comprises greater than about 80% by weight of one polymorphic form of the compound and less than about 20% by weight of other polymorphic forms of the compound, more preferably greater than about 90% by weight of one polymorphic form of the compound and less than about 10% by weight of the other polymorphic forms of the compound, even more preferably greater than about 95% by weight of one polymorphic form of the compound and less than about 5% by weight of the other polymorphic forms of the compound, and most preferably greater than about 97% by weight of one polymorphic forms of the compound and less than about 3% by weight of the other polymorphic forms of the compound.

### 5.2 Polymorphic Forms

This invention is directed to polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, which has the structure shown below:



This compound can be prepared according to the methods described in U.S. Pat. Nos. 6,281,230 and 5,635,517, the

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entireties of which are incorporated herein by reference. For example, the compound can be prepared through catalytic hydrogenation of 3-(4-nitro-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. 3-(4-Nitro-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can be obtained by allowing 2,6-dioxopiperidin-3-ammonium chloride to react with methyl 2-bromomethyl-4-nitrobenzoate in dimethylformamide in the presence of triethylamine. The methyl 2-bromomethyl-4-nitrobenzoate in turn is obtained from the corresponding methyl ester of nitro-ortho-toluic acid by conventional bromination with N-bromosuccinimide under the influence of light.

Polymorphs of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can be obtained by techniques known in the art, including solvent recrystallization, desolvation, vapor diffusion, rapid evaporation, slow evaporation, rapid cooling and slow cooling. Polymorphs can be made by dissolving a weighed quantity of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in various solvents at elevated temperatures. The solutions of the compound can then be filtered and allowed to evaporate either in an open vial (for fast hot evaporation) or in a vial covered with aluminum foil containing pinholes (hot slow evaporation). Polymorphs can also be obtained from slurries. Polymorphs can be crystallized from solutions or slurries using several methods. For example, a solution created at an elevated temperature (e.g., 60° C.) can be filtered quickly then allowed to cool to room temperature. Once at room temperature, the sample that did not crystallize can be moved to a refrigerator then filtered. Alternatively, the solutions can be crash cooled by dissolving the solid in a solvent at an increased temperature (e.g., 45-65° C.) followed by cooling in a dry ice/solvent bath.

One embodiment of the invention encompasses Form A of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form A is an unsolvated, crystalline material that can be obtained from non-aqueous solvent systems. Another embodiment of the invention encompasses Form B of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form B is a hemihydrated, crystalline material that can be obtained from various solvent systems. Another embodiment of the invention encompasses Form C of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form C is a hemisolvated crystalline material that can be obtained from solvents such as, but not limited to, acetone. Another embodiment of the invention encompasses Form D of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form D is a crystalline, solvated polymorph prepared from a mixture of acetonitrile and water. Another embodiment of the invention encompasses Form E of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form E is a dihydrated, crystalline material. Another embodiment of the invention encompasses Form F of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form F is an unsolvated, crystalline material that can be obtained from the dehydration of Form E. Another embodiment of the invention encompasses Form G of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form G is an unsolvated, crystalline material that can be obtained from slurrying forms B and E in a solvent such as, but not limited to, tetrahydrofuran (THF). Another embodiment of the invention encompasses Form H of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form H is a partially hydrated crystalline material that can be obtained by exposing Form E to 0% relative humidity. Each of these forms is discussed in detail below.

Another embodiment of the invention encompasses a composition comprising amorphous 3-(4-amino-1-oxo-1,3 dihy-

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dro-isoindol-2-yl)-piperidine-2,6-dione and crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of form A, B, C, D, E, F, G or H. Specific compositions can comprise greater than about 50, 75, 90 or 95 weight percent crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Another embodiment of the invention encompasses a composition comprising at least two crystalline forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione (e.g., a mixture of polymorph forms B and E).

## 5.2.1 Form A

The data described herein for Form A, as well as for Forms B-H, were obtained using the experimental methods described in Examples 6.3-6.7, provided below.

Form A can be obtained from various solvents, including, but not limited to 1-butanol, butyl acetate, ethanol, ethyl acetate, methanol, methyl ethyl ketone, and THF. FIG. 1 shows a representative XRPD pattern of Form A. The pattern is characterized by peaks, preferably significant peaks, at approximately 8, 14.5, 16, 17.5, 20.5, 24, and 26 degrees 2 $\theta$ . Representative IR and Raman spectra data are provided in FIGS. 2 and 3.

Representative thermal characteristics of Form A are shown in FIG. 4. TGA data show a small weight increase up to about 150° C., indicating an unsolvated material. Weight loss above 150° C. is attributed to decomposition. The DSC curve of Form A exhibits an endotherm at about 270° C.

Representative moisture sorption and desorption data are plotted in FIG. 5. Form A does not exhibit a significant weight gain from 5 to 95% relative humidity. Equilibrium can be obtained at each relative humidity step. As the form dries from 95% back down to 5% relative humidity, it tends to maintain its weight such that at 5% relative humidity it has typically lost only about 0.003% by weight from start to finish. Form A is capable of remaining a crystalline solid for about 11 days when stored at about 22, 45, 58, and 84% relative humidity.

Interconversion studies show that Form A can convert to Form B in aqueous solvent systems and can convert to Form C in acetone solvent systems. Form A tends to be stable in anhydrous solvent systems. In water systems and in the presence of Form E, Form A tends to convert to Form E.

When stored for a period of about 85 days under two different temperature/relative humidity stress conditions (room temperature/0% relative humidity (RH) and 40° C./93% RH), Form A typically does not convert to a different form.

In sum, Form A is a crystalline, unsolvated solid that melts at approximately 270° C. Form A is weakly or not hygroscopic and appears to be the most thermodynamically stable anhydrous polymorph of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione discovered thus far.

## 5.2.2 Form B

Form B can be obtained from many solvents, including, but not limited to, hexane, toluene, and water. FIG. 6 shows a representative XRPD pattern of Form B, characterized by peaks at approximately 16, 18, 22 and 27 degrees 2 $\theta$ .

Solution proton NMR confirm that Form B is a form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Representative IR and Raman spectra are shown in FIGS. 7 and 8, respectively. Compared to Form A, the IR spectrum for Form B has peaks at approximately 3513 and 1960 cm<sup>-1</sup>.

Representative DSC and TGA data for Form B are shown in FIG. 9. The DSC curve exhibits endotherms at about 146 and 268° C. These events are identified as dehydration and melting by hot stage microscopy experiments. Form B typi-



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cally loses about 3.1% volatiles up to about 175° C. (per approximately 0.46 moles of water). Comparison of the IR spectrum of the volatiles with that of water indicates that they are water (See FIG. 10). Calculations from TGA data indicate that Form B is a hemihydrate. Karl Fischer water analysis also

Representative moisture sorption and desorption data are shown in FIG. 11. Form B typically does not exhibit a significant weight gain from 5% to 95% relative humidity, when equilibrium is obtained at each relative humidity step. As Form B dries from 95% back down to 5% relative humidity, it tends to maintain its weight such that at 5% relative humidity it typically has gained only about 0.022% by weight (about 0.003 mg) from start to finish. Form B does not convert to a different form upon exposure to about 84% relative humidity for about ten days.

Interconversion studies show that Form B typically converts to Form A in a THF solvent system, and typically converts to Form C in an acetone solvent system. In aqueous solvent systems such as pure water and 10% water solutions, Form B is the most stable of the polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. However, it can convert to Form E in the presence of water. Desolvation experiments show that upon heating at about 175° C. for about five minutes, Form B typically converts to Form A.

When stored for a period of about 85 days under two different temperature/relative humidity stress conditions (room temperature/0% RH and 40° C./93% RH), Form B does not convert to a different form.

In sum, Form B is a hemihydrated, crystalline solid which has a DSC thermogram exhibiting endotherms at about 146 and about 268° C. Interconversion studies show that Form B converts to Form E in aqueous solvent systems, and converts to other forms in acetone and other anhydrous systems.

#### 5.2.3 Form C

Form C can be obtained from evaporations, slurries and slow cools in acetone solvent systems. A representative XRPD pattern of this form is shown in FIG. 12. The data are characterized by peaks at approximately 15.5 and 25 degrees 2 $\theta$ .

Solution proton NMR indicates that the 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione molecule is intact. Representative IR and Raman spectra are shown in FIGS. 13 and 14, respectively. The IR spectrum of Form C is characterized by peaks at approximately 3466, 3373, and 3318 cm<sup>-1</sup>. The Raman spectrum of Form C is characterized by peaks at about 3366, 3321, 1101, and 595 cm<sup>-1</sup>.

Representative thermal characteristics for Form C are plotted in FIG. 15. Form C loses about 10.02% volatiles up to about 175° C., indicating it is a solvated material. Weight loss above about 175° C. is attributed to decomposition. Identification of volatiles in Form C can be accomplished with TG-IR experiments. The representative IR spectrum captured after several minutes of heating, as depicted in FIG. 13, when compared with a spectral library, shows acetone to be the best match. Calculations from TGA data show that Form C is a hemisolvate (approximately 0.497 moles of acetone). The DSC curve for Form C, shown in FIG. 15, exhibits endotherms at about 150 and about 269° C. The endotherm at about 150° C. is attributed to solvent loss based on observations made during hot stage microscopy experiments. The endotherm at about 269° C. is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption balance data are shown in FIG. 17. Form C does not exhibit a significant weight gain from 5 to 85% relative humidity, when

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equilibrium is obtained at each relative humidity step up to 85% relative humidity. At 95% relative humidity, Form C experiences a significant weight loss of about 6.03%. As the sample dries from 95% back down to 5% relative humidity, the sample maintains the weight achieved at the end of the adsorption phase at each step down to 5% relative humidity. Form C is capable of converting to Form B when stored at about 84% relative humidity for approximately ten days.

Interconversion studies show that Form C typically converts to Form A in a THF solvent system and typically converts to Form E in an aqueous solvent system. In an acetone solvent system, Form C is the most stable form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Desolvation experiments performed on Form C show that upon heating at about 150° C. for about five minutes, Form C will typically convert to Form A.

In sum, Form C is a crystalline, hemisolvated solid, which has a DSC thermogram exhibiting endotherms at about 150 and about 269° C. Form C is not hygroscopic below about 85% RH, but can convert to Form B at higher relative humidities.

#### 5.2.4 Form D

Form D can be obtained from evaporation in acetonitrile solvent systems. A representative XRPD pattern of the form is shown in FIG. 18. The pattern is characterized by peaks at approximately 27 and 28 degrees 2 $\theta$ .

Solution proton NMR indicates that the 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione molecule is intact. Representative IR and Raman spectra are shown in FIGS. 19 and 20, respectively. The IR spectrum of Form D is characterized by peaks at approximately 3509, 2299, and 2256 cm<sup>-1</sup>. The Raman spectrum of Form D is characterized by peaks at approximately 2943, 2889, 2297, 2260, 1646, and 1150 cm<sup>-1</sup>.

Representative thermal characteristics for Form D are plotted in FIG. 21. Form D loses about 6.75% volatiles up to about 175° C., indicating a solvated material. Weight loss above about 175° C. is attributed to decomposition. TG-IR experiments indicate that the volatiles are water and acetonitrile. Calculations from TG data show that about one mole of water is present in the sample. A representative DSC curve for Form D exhibits endotherms at about 122 and about 270° C. The endotherm at about 122° C. is attributed to loss of volatiles based on observations made during hot stage microscopy experiments. The endotherm at about 270° C. is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption data are plotted in FIG. 22. Form D does not exhibit a significant weight gain from 5 to 95% relative humidity when equilibrium is obtained at each relative humidity step. As the form dries from 95% back down to 5% relative humidity, it maintains its weight such that at 5% relative humidity the form has typically gained only about 0.39% by weight (about 0.012 mg) from start to finish. Form A is capable of converting to Form B when stored at about 84% relative humidity for approximately ten days.

Interconversion studies show that Form D is capable of converting to Form A in a THF solvent system, to Form E in an aqueous solvent system, and to Form C in an acetone solvent system. Desolvation experiments performed on Form D show that upon heating at about 150° C. for about five minutes Form D will typically convert to Form A.

In sum, Form D is a crystalline solid, solvated with both water and acetonitrile, which has a DSC thermogram exhibiting endotherms at about 122 and about 270° C. Form D is either weakly or not hygroscopic, but will typically convert to Form B when stressed at higher relative humidities.



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## 5.2.5 Form E

Form E can be obtained by slurrying 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in water and by a slow evaporation of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in a solvent system with a ratio of about 9:1 acetone:water. A representative XRPD pattern is shown in FIG. 23. The data are characterized by peaks at approximately 20, 24.5 and 29 degrees 2 $\theta$ .

Representative thermal characteristics of Form E are plotted in FIG. 24. Form E typically loses about 10.58% volatiles up to about 125° C., indicating that it is a solvated material. A second weight loss of an additional about 1.38% was observed between about 125° C. and about 175° C. Weight loss above about 175° C. is attributed to decomposition. Karl Fischer and TG-IR experiments support the conclusion that the volatile weight loss in Form E is due to water. The representative DSC curve for Form E exhibits endotherms at about 99, 161 and 269° C. Based on observations made during hot stage microscopy experiments, the endotherms at about 99 and about 161° C. are attributed to loss of volatiles. The endotherm at about 269° C. is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption data are plotted in FIG. 25. Form E typically does not exhibit a significant weight change from 5 to 95% relative humidity when equilibrium is obtained at each relative humidity step. As the sample dried from 95% back down to 5% relative humidity, the sample continues to maintain weight such that at 5% relative humidity the sample has lost only about 0.0528% by weight from start to finish.

Interconversion studies show that Form E can convert to Form C in an acetone solvent system and to Form G in a THF solvent system. In aqueous solvent systems, Form E appears to be the most stable form. Desolvation experiments performed on Form E show that upon heating at about 125° C. for about five minutes, Form E can convert to Form B. Upon heating at 175° C. for about five minutes, Form B can convert to Form F.

When stored for a period of 85 days under two different temperature/relative humidity stress conditions (room temperature/0% RH and 40° C./93% RH) Form E typically does not convert to a different form. When stored for seven days at room temperature/0% RH, Form E can convert to a new form, Form H.

## 5.2.6 Form F

Form F can be obtained by complete dehydration of Form E. A representative XRPD pattern of Form F, shown in FIG. 26, is characterized by peaks at approximately 19, 19.5 and 25 degrees 2 $\theta$ .

Representative thermal characteristics of Form F are shown in FIG. 27. The representative DSC curve for Form F exhibits an endotherm at about 269° C. preceded directly by two smaller endotherms indicative of a crystallized form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The DSC thermogram does not show any thermal events prior to the melt, suggesting that it is an unsolvated material.

## 5.2.7 Form G

Form G can be obtained by slurrying forms B and E in THF. A representative XRPD pattern of this form, shown in FIG. 28, is characterized by a peak at approximately 23 degrees 2 $\theta$ . Two other peaks unique to Form G appear at approximately 21 and 24.5 degrees 2 $\theta$ .

Representative thermal characteristics of Form G are plotted in FIG. 29. A representative DSC curve for Form G exhibits an endotherm at about 248° C. followed by a small, broad exotherm at about 267° C. No thermal events are seen

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in the DSC thermogram at lower temperatures, suggesting that it is an unsolvated material.

## 5.2.8 Form H

Form H can be obtained by storing Form E at room temperature and 0% RH for about 7 days. A representative XRPD pattern is shown in FIG. 30. The pattern is characterized by a peak at 15 degrees 2 $\theta$ , and two other peaks at 26 and 31 degrees 2 $\theta$ .

Representative thermal characteristics are shown in FIG. 31. Form H loses about 1.67% volatiles up to about 150° C. Weight loss above about 150° C. is attributed to decomposition. Karl Fischer data shows that Form H typically contains about 1.77% water (about 0.26 moles), suggesting that the weight loss seen in the TG is due to dehydration. The DSC thermogram shows a broad endotherm between about 50° C. and about 125° C., corresponding to the dehydration of Form H and a sharp endotherm at about 269° C., which is likely due to a melt.

When slurried in water with either Forms A or B, after about 14 days Form H can convert to Form E. When slurried in THF, Form H can convert to Form A. When slurried in acetone, Form H can convert to Form C.

In sum, Form H is a crystalline solid, hydrated with about 0.25 moles of water, which has a DSC thermogram exhibiting an endotherm between about 50 and 125° C. and an endotherm at about 269° C.

## 5.3 Methods of Use and Pharmaceutical Compositions

Polymorphs of the invention exhibit physical characteristics that are beneficial for drug manufacture, storage or use. All polymorphs of the invention have utility as pharmaceutically active ingredients or intermediates thereof.

This invention encompasses methods of treating and preventing a wide variety of diseases and conditions using polymorphs of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. In each of the methods, a therapeutically or prophylactically effective amount of the compound is administered to a patient in need of such treatment or prevention. Examples of such disease and conditions include, but are not limited to, diseases associated with undesired angiogenesis, cancer (e.g., solid and blood borne tumors), inflammatory diseases, autoimmune diseases, and immune diseases. Examples of cancers and pre-cancerous conditions include those described in U.S. Pat. Nos. 6,281,230 and 5,635,517 to Muller et al. and in various U.S. patent applications to Zeldis, including application Ser. No. 10/411,649, filed Apr. 11, 2003 (Treatment of Myelodysplastic Syndrome); Ser. No. 10/438,213 filed May 15, 2003 (Treatment of Various Types of Cancer); Ser. No. 10/411,656, filed Apr. 11, 2003 (Treatment of Myeloproliferative Diseases). Examples of other diseases and disorders that can be treated or prevented using compositions of the invention are described in U.S. Pat. Nos. 6,235,756 and 6,114,335 to D'Amato and in other U.S. patent applications to Zeldis, including Ser. No. 10/693,794, filed Oct. 23, 2003 (Treatment of Pain Syndrome) and Ser. No. 10/699,154, filed Oct. 30, 2003 (Treatment of Macular Degeneration). The entirety of each of the patents and patent applications cited herein is incorporated herein by reference.

Depending on the disease to be treated and the subject's condition, polymorphs of the invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implantation), inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes of administration and may be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration. Because individual poly-

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morphs have different dissolution, stability, and other properties, the optimal polymorph used in methods of treatment may depend on the route of administration. For example, forms that are readily soluble in aqueous solutions are preferably used to provide liquid dosage forms, whereas forms that exhibit great thermal stability may be preferred in the manufacture of solid dosage forms (e.g., tablets and capsules).

Although the physical characteristics of polymorphs can, in some cases, affect their bioavailability, amounts of the polymorphs that are therapeutically or prophylactically effective in the treatment of various disease and conditions can be readily determined by those of ordinary skill in the pharmacy or medical arts. In certain embodiments of the invention, a polymorph is administered orally and in a single or divided daily doses in an amount of from about 0.10 to about 150 mg/day, or from about 5 to about 25 mg/day. In other embodiments, a polymorph is administered every other day in an amount of from about 0.10 to about 150 mg/day, or from about 5 to about 25 mg/day.

The invention encompasses pharmaceutical compositions and single unit dosage forms that can be used in methods of treatment and prevention, which comprise one or more polymorphs of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione and optionally one or more excipients or diluents. Specific compositions and dosage forms are disclosed in the various patents and patent applications incorporated herein by reference. In one embodiment, a single dosage form comprises a polymorph (e.g., Form B) in an amount of about 5, 10, 25 or 50 mg.

## 6. EXAMPLES

### 6.1 Polymorph Screen

A polymorph screen to generate the different solid forms of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione was carried out as follows.

A weighed sample of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (usually about 10 mg) was treated with aliquots of the test solvent. Solvents were either reagent or HPLC grade. The aliquots were usually about 200  $\mu$ L. Between additions, the mixture was usually shaken or sonicated. When the solids dissolved, as judged by visual inspection, estimated solubilities were calculated. Solubilities were estimated from these experiments based on the total solvent used to provide a solution. Actual solubilities may have been greater than those calculated due to the use of too-large solvent aliquots or to a slow rate of dissolution.

Samples were created by generating solutions (usually about 30 mg in 20 mL) at elevated temperatures, filtering, and allowing the solution to evaporate whether in an open vial (hot fast evaporation) or in a vial covered with aluminum foil containing pinholes (hot slow evaporation).

Slurry experiments were also performed. Usually about 25 mg of solid was placed in either 3 or 5 mL of solvent. The samples were then placed on orbital shakers at either ambient temperature or 40° C. for 4-10 days.

Crystallizations were performed using various cooling methods. Solid was dissolved in a solvent at an elevated temperature (e.g., about 60° C.), filtered quickly and allowed to cool to room temperature. Once at room temperature, samples that did not crystallize were moved to a refrigerator. Solids were removed by filtration or decantation and allowed to dry in the air. Crash cools were performed by dissolving solid in a solvent at an increased temperature (e.g., about 45-65° C.) followed by cooling in a dry ice/acetone bath.

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Hygroscopicity studies were performed by placing portions of each polymorph in an 84% relative humidity chamber for approximately one week.

Desolvation studies were carried out by heating each polymorph in a 70° C. oven for approximately one week.

Interconversion experiments were carried out by making slurries containing two forms in a saturated solvent. The slurries were agitated for approximately 7-20 days at ambient temperature. The insoluble solids were recovered by filtration and analyzed using XRPD.

### 6.2 Preparation of Polymorphic Forms

Eight solid forms of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione were prepared as described below.

Form A was obtained by crystallization from various non-aqueous solvents including 1-butanol, butyl acetate, ethanol, ethyl acetate, methanol, methyl ethyl ketone, and tetrahydrofuran. Form B was also obtained by crystallization from the solvents hexane, toluene and water. Form C was obtained from evaporations, slurries, and slow cools in acetone solvent systems. Form D was obtained from evaporations in acetonitrile solvent systems. Form E was obtained most readily by slurrying 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in water. Form F was obtained by complete desolvation of Form E. It is found to be an unsolvated, crystalline material that melts at about 269° C. Form G was obtained by slurrying forms B and E in THF. Form H was obtained by stressing Form E at room temperature and 0% RH for 7 days.

#### 6.2.1 Synthesis of Polymorphs B and E

Form B is the desired polymorph for the active pharmaceutical ingredient (API) of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. This form has been used in the formulation of API into drug product for clinical studies. Three batches were produced as apparent mixtures of polymorphs in the non-micronized API of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Development work was carried out to define a process that would generate polymorph B from this mixture of polymorphs and could be implemented for strict polymorphic controls in the validation batches and future manufacturing of API of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Characterization of polymorphic forms produced during the work was performed by XRPD, DSC, TGA and KF.

A process was also developed for the large-scale preparation of Form E. Polymorph E material was prepared in order to carry out a comparison with polymorph B drug product in capsule dissolution testing of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. 150 g of a mixture of polymorphs in 3 L of water was stirred at room temperature for 48 hours. The product was collected by filtration and dried at 25° C. for 24 hours under vacuum. XRPD, DSC, TGA, KF and HPLC analyses confirmed that the material isolated was polymorph E.

In a preliminary work, it was demonstrated that stirring a suspension of a mixture of polymorphs of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione with water at high temperature (75° C.) for an extended period of time converted this mixture of polymorphs exclusively to form B. Several specific parameters were identified including temperature, solvent volume and drying parameters (temperature and vacuum). XRPD, DSC, TGA, KF and HPLC analyses were used to characterize all of the batches. After completing the optimization work, the optimized process was scaled-up to 100-200 g on three lots of API. Drying studies were carried

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out at 20° C., 30° C. and 40° C., and 65° C. with a vacuum of 150 mm of Hg. The results are shown in Tables 1-5.

The cooling and holding periods of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione slurry were studied. The experimental laboratory data suggests that polymorph B seems to be forming first, and overtime equilibration to polymorph E at RT conditions occurs, therefore generating a mixture of polymorphs B and E. This result supports the fact that polymorph B seems to be a kinetic product, and that prolonged processing time converts the material to polymorph E resulting in a mixture of polymorphs B and E.

A laboratory procedure was developed to exclusively produce polymorph B of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The procedure includes a stirred 10 volume water slurry at ~75° C. for 6-24 hours. The following preferred process parameters have been identified:

1. Hot slurry temperature of 70-75° C.
2. Product filtration of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione at 65-75° C.
3. Drying under vacuum at 60-70° C. is preferred for an efficient removal of unbound water in 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione wet cake.
4. The filtration step of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione may be a time sensitive operation. The use of efficient solid-liquid separation equipment is preferred.
5. Holding periods of water-wet cake of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione at KF higher than 5% may cause the kinetic equilibrations of polymorph B to mixed polymorphs of E and B.

Drying to KF <4.0% water was achieved in ~3 hours (30-70° C., 152 mm Hg). Polymorphs B and E were distinguished by the water levels as measured by KF and TGA. The reference sample of polymorph B is micronized API. In order to make accurate comparison by XRPD samples were gently grinded before submission for analysis. This increases the clarity of the identification of the polymorphic form. All samples were analyzed for XRPD, DSC, TGA, KF and HPLC.

TABLE 1

Preliminary Studies			
Amount	Reaction conditions	Analysis	Results/conclusion
2 g	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
25 g	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
5 g	Water, 70-75° C., 24 h then rt 24 h	XRPD, DSC, TGA, KF	Polymorph B
1 g	9:1 Acetone - water, Slow evpo. 175° C. 1 h in an oven	XRPD, DSC, TGA, KF	Polymorph Mixture
1 g	Water, rt, 24 h	XRPD, DSC, TGA, KF	Polymorph A
0.5 g (polymorph A)	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
1 g polymorph B	Water, 70-75° C., 24 h	XRPD, DSC, TGA, KF	Polymorph B
1 g polymorph E	Slurry in heptane	XRPD, DSC, TGA, KF	No change

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TABLE 2

Optimization of Temperature, Time and Solvent Volume				
Amount	Amount Water (mL)	Temp (° C.)	Time (h)	Results/conclusion
10 g	50	75	6	Mix
10 g	50	75	24	Polymorph B
10 g	100	70	6	Polymorph B
10 g	100	70	14	Polymorph B
10 g	100	70	21	Polymorph B
10 g	100	75	6	Polymorph B
10 g	100	75	24	Polymorph B
10 g	100	75	6	Polymorph B
10 g	100	75	19	Polymorph B
10 g	100	75	14	Polymorph B
10 g	100	75	24	Polymorph B
5 g	100	75	18	Polymorph B
10 g	100	80	6	Polymorph B
10 g	100	80	20	Polymorph B
10 g	200	45	6	Polymorph B + E
10 g	200	45	24	Polymorph E
10 g	200	60	48	Polymorph B
10 g	200	75	6	Mix
10 g	200	75	24	Polymorph B
10 g	200	75	13	Polymorph B
10 g	200	75	24	Polymorph B

Optimum conditions were determined to be 10 volumes of solvent (H<sub>2</sub>O), 70-80° C. for 6-24 hours.

TABLE 3

Holding Time				
Amount	Reaction Conditions	Holding Time (h)	Holding Temp (° C.)	Results/Conclusion
5 g	Water, 70-75° C., 24 h	24	23-25	Polymorph B
1 g	Water, 70-75° C., 24 h	48	23-25	Polymorph E
Polymorph B				
2 g	Water, 40 mL	16	23-25	Polymorph E
150 g	Water, 3.0 L	24	23-25	Polymorph E
150 g	Water, 3.0 L	48	23-25	Polymorph E
10 g	Water, 100 mL, 24 h, 75° C.	18	23-25	Polymorph B
10 g	Water, 100 mL, 24 h, 75° C.	18	40	Polymorph B
10 g	Water, 200 mL, 24 h, 75° C.	14	-5	Mix
10 g	Water, 200 mL, 24 h, 75° C.	14	23-25	Polymorph E
10 g	Water, 200 mL, 24 h, 75° C.	14	40	Mix
10 g	Water, 100 mL, 24 h, 75° C.	21	23-25	Polymorph E
10 g	Water, 100 mL, 24 h, 75° C.	21	40	Mix
10 g	Water, 100 mL, 14 h, 75° C.	2	23-25	Mix

Holding time gave mixed results and it was determined that the material should be filtered at 60-65° C. and the material washed with 0.5 volume of warm (50-60° C.) water.

TABLE 4

Scale-up Experiments				
Amount	Amount Water (L)	Temp (° C.)	Time (h)	Results/Conclusion
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	22	Polymorph B

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TABLE 4-continued

Scale-up Experiments				
Amount	Amount Water (L)	Temp (° C.)	Time (h)	Results/ Conclusion
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	24	Polymorph B
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	22	Polymorph B

TABLE 5

Drying Studies					
Amount	Drying Time (h)	Drying Temp (° C.)	Vacuum (mm Hg)	KF§ (%)	Results/ Conclusion
100 g	0	—	—	3.690	Polymorph B
100 g	3	30	152	3.452	Polymorph B
100 g	8	30	152	3.599	Polymorph B
100 g	0	—	—	3.917	Polymorph B
100 g	5	40	152	3.482	Polymorph B
100 g	22	40	152	3.516	Polymorph B
100 g	3	40	152	3.67	Polymorph B
100 g	22	40	152	3.55	Polymorph B

\* Reaction Conditions: Water 1 L, 75° C., 22-24 h;

§ Average of 2 runs.

Drying studies determined that the material should be dried at 35-40° C., 125-152 mm Hg for 3 to 22 h or until the water content reaches  $\leq 4\%$  w/w.

For a large scale preparation of polymorph E (5222-152-B), a 5-L round bottom flask was charged with 3-(4-amino-1-oxo-1,3 dihydro-isoinol-2-yl)-piperidine-2,6-dione (150 g, 0.579 mol) and water (3000 mL, 20 vol). The mixture was mechanically stirred at room temperature (23-25° C.) for 48 h under nitrogen atmosphere.

Samples were taken after 24 h and 48 h before the mixture was filtered and air-dried on the filter for 1 h. The material was transferred to a drying tray and dried at room temperature (23-25° C.) for 24 h. KF analysis on the dried material showed water content of 11.9%. The material was submitted for XRPD, TGA, DSC and HPLC analysis. Analysis showed the material was pure polymorph E.

For a large scale preparation of polymorph B (5274-104), a 2 L-3-necked round bottom flask was charged with 3-(4-amino-1-oxo-1,3 dihydro-isoinol-2-yl)-piperidine-2,6-dione (polymorph mixture, 100 g, 0.386 mol) and water (1000 mL, 10.0 vol). The mixture was heated to 75° C. over approximately 30 minutes with mechanical stirring under nitrogen atmosphere.

Samples were taken after 6 h and 24 h before the mixture was allowed to cool to 60-65° C., filtered and the material washed with warm (50-60° C.) water (50 mL, 0.5 vol). The material was transferred to a drying tray and dried at 30° C., 152 mm Hg for 8 h. KF analysis on the dried material showed water content of 3.6%. After grinding the material was submitted for XRPD, TGA, DSC and HPLC analysis. Analysis showed the material was pure polymorph B. The results of the analyses are shown in FIGS. 32-46.

### 6.3 X-Ray Powder Diffraction Measurements

X-ray powder diffraction analyses were carried out on a Shimadzu XRD-6000 X-ray powder diffractometer using Cu K $\alpha$  radiation. The instrument is equipped with a fine-focus X-ray tube. The tube voltage and amperage were set at 40 kV and 40 mA, respectively. The divergence and scattering slits were set at 1° and the receiving slit was set at 0.15 mm.

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Diffacted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3°/min (0.4 sec/0.02° step) from 2.5 degrees 2 $\theta$  to 40 degrees 2 $\theta$  was used. A silicon standard was analyzed each day to check the instrument alignment.

X-ray powder diffraction analyses were also carried out using Cu K $\alpha$  radiation on an Inel XRG-3000 diffractometer equipped with a curved position-sensitive detector. Data were collected in real time over a theta-two theta range of 120° at a resolution of 0.030°. The tube voltage and current were 40 kV and 30 mA, respectively. A silicon standard was analyzed each day to check for instrument alignment. Only the region between 2.5 and 40 degrees 2 $\theta$  is shown in the figures.

### 6.4 Thermal Analysis

TG analyses were carried out on a TA Instrument TGA 2050 or 2950. The calibration standards were nickel and alumel. Approximately 5 mg of sample was placed on a pan, accurately weighed, and inserted into the TG furnace. The samples were heated in nitrogen at a rate of 10° C./min, up to a final temperature of 300 or 350° C.

DSC data were obtained on a TA 2920 instrument. The calibration standard was indium. Approximately 2-5 mg samples were placed into a DSC pan and the weight accurately recorded. Crimped pans with one pinhole were used for analysis and the samples were heated under nitrogen at a rate of 10° C./min, up to a final temperature of 350° C.

Hot-stage microscopy was carried out using a Kofler hot stage mounted on a Leica Microscope. The instrument was calibrated using USP standards.

A TA Instruments TGA 2050 interfaced with a Nicolet model 560 Fourier transform IR spectrophotometer, equipped with a globar source, XT/KBr beamsplitter, and deuterated triglycine sulfate (DTGS) detector, was utilized for TG-IR experiments. The IR spectrometer was wavelength calibrated with polystyrene on the day of use while the TG was temperature and weight calibrated biweekly, using indium for the temperature calibration. A sample of approximately 10 mg of 3-(4-amino-1-oxo-1,3 dihydro-isoinol-2-yl)-piperidine-2,6-dione was weighed into an aluminum pan and heated from 25 to 30° C. to 200° C. at a rate of 20° C./min with a helium purge. IR spectra were obtained in series, with each spectrum representing 32 co-added scans at a resolution of 4 cm<sup>-1</sup>. Spectra were collected with a 17-second repeat time. TG/IR analysis data are presented as Gram-Schmidt plots and IR spectra linked to the time. Gram-Schmidt plots show total IR intensity vs. time; hence, the volatiles can be identified at each time point. They also show when the volatiles are detected. From the Gram-Schmidt plots, time points were selected and the IR spectra of these time points are presented in the stacked linked spectra. Each spectrum identifies volatiles evolving at that time point. Volatiles were identified from a search of the HR Nicolet TGA vapor phase spectral library. The library match results are also presented to show the identified vapor.

### 6.5 Spectroscopy Measurements

Raman spectra were acquired on a Nicolet model 750 Fourier transform Raman spectrometer utilizing an excitation wavelength of 1064 nm and approximately 0.5 W of Nd:YAG laser power. The spectra represent 128 to 256 co-added scans acquired at 4 cm<sup>-1</sup> resolution. The samples were prepared for analysis by placing the material in a sample holder and positioning this in the spectrometer. The spectrometer was wavelength calibrated using sulfur and cyclohexane at the time of use.

The mid-IR spectra were acquired on a Nicolet model 860 Fourier transform IR spectrophotometer equipped with a glo-



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bar source XT/KBr beamsplitter and a deuterated triglycine sulfate (DTGS) detector. A Spectra-Tech, Inc. diffuse reflectance accessory was utilized for sampling. Each spectrum represents 128 co-added scans at a spectral resolution of 4  $\text{cm}^{-1}$ . A background data set was acquired with an alignment mirror in place. A single beam sample data set was then acquired. Subsequently, a log 1/R (where R=reflectance) spectrum was acquired by rationing the two data sets against each other. The spectrophotometer was calibrated (wavelength) with polystyrene at the time of use.

#### 6.6 Moisture Sorption/Desorption Measurements

Moisture sorption/desorption data were collected on a VTI SGA-100 moisture balance system. For sorption isotherms, a sorption range of 5 to 95% relative humidity (RH) and a desorption range of 95 to 5% RH in 10% RH increments was used for analysis. The sample was not dried prior to analysis. Equilibrium criteria used for analysis were less than 0.0100 weight percent change in 5 minutes with a maximum equilibration time of 3 hours if the weight criterion was not met. Data were not corrected for the initial moisture content of the samples.

#### 6.7 Solution Proton NMR Measurements

NMR spectra not previously reported were collected at SSCI, Inc, 3065 Kent Avenue, West Lafayette, Ind. Solution phase  $^1\text{H}$  NMR spectra were acquired at ambient temperature on a Bruker model AM spectrometer. The  $^1\text{H}$  NMR spectrum represents 128 co-added transients collected with a 4  $\mu\text{sec}$  pulse and a relaxation delay time of 5 seconds. The free induction decay (FID) was exponentially multiplied with a 0.1 Hz Lorentzian line broadening factor to improve the signal-to-noise ratio. The NMR spectrum was processed utilizing GRAMS software, version 5.24. Samples were dissolved in dimethyl sulfoxide- $d_6$ .

The scope of this invention can be understood with reference to the appended claims.

#### 6.8 Intrinsic Dissolution and Solubility Studies

Intrinsic dissolution experiments were conducted on Form A (anhydrous), Form B (hemihydrate), and Form E (dihydrate) of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Equilibrium solubility experiments were conducted on Forms A and B. Aliquots were analyzed by ultraviolet-visible spectrophotometry, and the solids remaining from each experiment were analyzed by X-ray powder diffraction (XRPD).

##### 6.8.1 Experimental

##### 6.8.1.1 Dissolution

Dissolution experiments were carried out in a VanKel VK6010-8 dissolution apparatus equipped with a VK650A heater/circulator. An intrinsic dissolution apparatus (Woods apparatus) was used. Samples were compressed at 1.5 metric tons (1000 psi) for 1 min using the Woods apparatus in a hydraulic press, giving a sample surface of 0.50  $\text{cm}^2$ . A dissolution medium consisting of 900 mL HCl buffer, pH 1.8, with 1% sodium lauryl sulfate, was used for each experiment. The medium was degassed by vacuum filtration through a 0.22- $\mu\text{m}$  nylon filter disk and maintained at 37° C. The apparatus was rotated at 50 rpm for each experiment. Aliquots were filtered immediately using 0.2- $\mu\text{m}$  nylon syringe filters. In some cases, the undissolved solids were recovered and analyzed by X-ray powder diffraction (XRPD).

##### 6.8.1.2 Solubility

Equilibrium solubility experiments were conducted in a 100-mL, three-neck, round-bottom flask immersed in a constant temperature oil bath maintained at 25° C. A solid sample of 400-450 mg was stirred in 50 mL of dissolution medium (HCl buffer, pH 1.8, with 1% sodium lauryl sulfate) using a mechanical stir rod. Aliquots were filtered using 0.2- $\mu\text{m}$

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nylon syringe filters and immediately diluted 1 mL→50 mL, then 5 mL→25 mL with dissolution medium in Class A glassware, a final dilution factor of 250.

##### 6.8.1.3 UV-Vis Spectrophotometry

Dissolution and solubility samples solutions were analyzed by a Beckman DU 640 single-beam spectrophotometer. A 1.000-cm quartz cuvette and an analysis wavelength of 228.40 nm were utilized. The detector was zeroed with a cuvette filled with dissolution medium.

##### 6.8.1.4 X-Ray Powder Diffraction

XRPD analyses were carried out on a Shimadzu XRD-6000 X-ray powder diffractometer using Cu K $\alpha$  radiation. The instrument is equipped with a fine focus X-ray tube. The tube power and amperage were set at 40 kV and 40 mA, respectively. The divergence and scattering slits were set at 1° and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3°/min (0.4 sec/0.02° step) from 2.5 to 40° 2 $\theta$  was used. A silicon standard was analyzed each day to check the instrument alignment. Samples were packed in an aluminum holder with silicon insert.

##### 6.8.2 Results

The results of these solubility and intrinsic studies are summarized in Table 6. Both the solubility and dissolution experiments were conducted in a medium of HCl buffer, pH 1.8, containing 1% sodium lauryl sulfate. Form A was found to be unstable in the medium, converting to Form E. The solubilities of Forms A, B, and E were estimated to be 6.2, 5.8, and 4.7 mg/mL, respectively. The dissolution rates of Forms A, B, and E were estimated to be 0.35, 0.34, and 0.23 mg/mL, respectively.

##### 6.8.2.1 UV-Vis Spectrophotometry Method Development

A UV-Vis scan of the dissolution medium (blanked with an empty cuvette) was done to identify any interfering peaks. A small peak at 225 nm was present as shown in FIG. 47.

Solutions of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione at varying concentrations were analyzed by UV-Vis spectrophotometry. A preliminary scan of a 1.0 mg/mL solution was done, with the instrument blanked with dissolution medium. The solution was highly absorbing and noisy from 200-280 nm, making dilution necessary.

A 0.04 mg/mL solution of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione was then scanned from 200-300 nm. The plot was still noisy between 200 and 230 nm as shown in FIG. 48. The sample was further diluted to 0.008 mg/mL. A wavelength scan of 200-350 nm for this sample showed a peak a 228.4 nm with no interference, as shown in FIG. 49. Therefore, a wavelength of 228.4 was chosen for analysis of the solubility and dissolution samples.

A six-point calibration curve was generated with standards of the following concentrations: 0.001 mg/mL, 0.002 mg/mL, 0.005 mg/mL, 0.010 mg/mL, 0.015 mg/mL, and 0.020 mg/mL (Notebook 569-90). A linearity coefficient of  $R^2=0.9999$  was obtained as shown in FIG. 50.

##### 6.8.2.2 Solubility

A sample consisting of 449.4 mg Form A was slurried in dissolution medium. Particle size was not controlled. Aliquots were taken at 7, 15, 30, 60, 90, and 150 min. The concentration reached 6.0 mg/mL by the first time point. The highest concentration reached was 6.2 mg/mL, at 30 min. From that point the concentration decreased, reaching 4.7 mg/mL at 150 min as in FIG. 51. The solids remaining at the final time point were analyzed by XRPD and found to be Form E as shown in Table 7. No peaks attributed to Form A can be seen in the pattern. Since the concentration did not plateau at 4.7 mg/mL, the solubility of Form E may be lower than that.

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A sample consisting of 401.4 mg Form B was slurried in dissolution medium. Particle size was not controlled. Aliquots were taken at 7, 15, 30, 60, 90, 180, 420, and 650 min. Form B dissolved much more slowly than Form A, reaching 3.3 mg/mL in 90 min. The concentration stabilized at 5.6-5.7 mg/mL at the final three time points as in FIG. 52. The remaining solids were shown to be Form B as in Table 7, suggesting Form B has good stability in water.

A summary of the solubilities is given in Table 6. The amounts dissolved at each time point are shown in Tables 8 and 9.

TABLE 6

Summary of Results				
Form	Solubility	Intrinsic Dissolution #1	Intrinsic Dissolution #2	Average Intrinsic Dissolution Rate
Form A	6.2 mg/mL	0.35	0.22 <sup>a</sup>	0.29 <sup>a</sup>
Form B	5.8 mg/mL	0.35	0.32	0.34
Form E	4.7 mg/mL	0.21	0.25	0.23

<sup>a</sup>The Form A dissolution experiment #2 may have converted to Form E on the surface of the disk, skewing the average rate lower.

TABLE 7

Experimental Details		Final Form
Experiment		
Pressed Form A		A
Pressed Form B		B
Form A Solubility		E
Form B Solubility		B
Form A Dissolution		—
Form A Dissolution		A
Form B Dissolution		—
Form B Dissolution		B
Form E Dissolution		E
Form E Dissolution		—

TABLE 8

Form A Solubility	
Time Point (min)	Concentration (mg/mL)
7	6.00
15	6.11
30	6.16
60	6.10
90	5.46
150	4.73

TABLE 9

Form B Solubility	
Time Point (min)	Concentration (mg/mL)
7	1.63
15	2.14
30	2.33
60	2.94
90	3.34
180	5.67
420	5.76
650	5.61

## 20

## 6.8.2.3 Intrinsic Dissolution

Approximately 200 mg each of Forms A and B were compressed into disks in the Woods apparatus using 2 metric tons of pressure. The samples were subsequently scraped out, ground gently, and analyzed by XRPD. The study showed that compression and grinding does not cause a form change in either case. (See Table 7).

Two preliminary dissolution runs were performed. The disks fractured to some extent in both experiments, compromising the requirement of constant surface area.

The first experiment of intrinsic dissolution that strictly followed the USP chapter on intrinsic dissolution utilized approximately 150 mg each of Forms A and B. Seven aliquots, beginning at 5 min and ending at 90 min, were taken to maintain sink conditions. The experiment resulted in linear dissolution profiles, with a rate of 0.35 mg per cm<sup>2</sup> per minute for both forms. The Form E experiment was done later under the same conditions and added to the graph for comparison. (See FIG. 53). The Form E dissolution rate was 0.21 mg per cm<sup>2</sup> per minute, significantly lower than the dissolution rate of Forms A and B. This is in line with expectations based on the solubility data. The crystal form of the remaining solids did not change in any case.

The second experiment utilized approximately 250 mg each of Forms A and B. The Form E experiment (135 mg) was done later and added to the graph for comparison. (See FIG. 54). Nine aliquots were taken, beginning at 5 min and ending at 150 min. The dissolution rates were 0.22, 0.32, and 0.25 mg per cm<sup>2</sup> per minute, respectively, for Forms A, B, and E. The dissolution rate for Form A in this experiment was low, while the rates for Forms B and E were similar to those found in the first experiment. It is believed that in this case, a thin layer of the Form A sample disk may have converted to Form E upon exposure to water. This is supported by the evidence of rapid conversion of Form A to Form E in the solubility experiment. The diffraction pattern of the undissolved solids does not indicate a form change. However, the bulk of the sample disk is not exposed to water. Therefore, the true intrinsic dissolution rate of Form A is believed to be close to 0.35 mg per cm<sup>2</sup> per minute. An insufficient quantity of Form A was available to repeat the experiment.

A summary of the intrinsic dissolution rates is given in Table 6. The amounts dissolved at each time point are summarized in Tables 10 and 11.

TABLE 10

Intrinsic Dissolution Experiment #1 Results				
Time Point	Form A <sup>a</sup>	Form B <sup>a</sup>	Form E <sup>a</sup>	
5 min	5.76	10.80 <sup>b</sup>	2.70	
10 min	7.73	6.85	4.13	
20 min	11.31	10.25	6.96	
30 min	15.59	14.35	9.60	
45 min	21.98	20.57	12.57	
60 min	27.11	25.70	15.16	
90 min	34.17	34.34	20.82	

<sup>a</sup>Results are reported as Cumulative Amount Dissolved per Unit Area (mg/cm<sup>2</sup>)

<sup>b</sup>This date point not included in graph since the value is higher than the next two data points.

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TABLE 11

Intrinsic Dissolution Experiment #2 Results			
Time Point	Form A <sup>a</sup>	Form B <sup>a</sup>	Form E <sup>a</sup>
5 min	4.50	5.04	3.06
10 min	5.22	6.12	4.31
20 min	7.54	7.73	11.40
30 min	11.46	12.72	11.93
45 min	15.01	17.33	14.72
60 min	18.38	21.93	18.52
90 min	24.38	31.64	26.24
120 min	30.35	41.31	33.56
150 min	35.26	49.54	40.82

<sup>a</sup>Results are reported as Cumulative Amount Dissolved per Unit Area (mg/cm<sup>2</sup>)

#### 6.9 Analyses of Mixtures of Polymorphs

This invention encompasses mixtures of different polymorphs. For example, an X-ray diffraction analysis of one production sample yielded a pattern that contained two small peaks seen at approximately 12.6° and 25.8° 2θ in addition to those representative of Form B. In order to determine the composition of that sample, the following steps were performed:

- 1) Matching of the new production pattern to known forms along with common pharmaceutical excipients and contaminants;
- 2) Cluster analysis of the additional peaks to identify if any unknown phase is mixed with the original Form B;
- 3) Harmonic analysis of the additional peaks to identify if any preferred orientation may be present or if any changes in the crystal habit may have occurred; and
- 4) Indexing of the unit cells for both Form B and the new production sample to identify any possible crystallographic relationships.

Based on these tests, which can be adapted for the analysis of any mixture of polymorphs, it was determined that the sample contained a mixture of polymorph forms B and E.

#### 6.10 Dosage Form

Table 12 illustrates a batch formulation and single dosage formulation for a 25 mg single dose unit of a polymorphic form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

TABLE 12

Formulation for a 25 mg capsule			
Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Polymorphic Form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione	40.0%	25 mg	16.80 kg
Pregelatinized Corn Starch, NF	59.5%	37.2 mg	24.99 kg
Magnesium Stearate	0.5%	0.31 mg	0.21 kg
Total	100.0%	62.5 mg	42.00 kg

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The pregelatinized corn starch (SPRESS B-820) and polymorphic form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione components are passed through a screen (i.e., a 710 μm screen) and then loaded into a Diffusion Mixer with a baffle insert and blended for about 15 minutes. The magnesium stearate is passed through a screen (i.e., a 210 μm screen) and added to the Diffusion Mixer. The blend is then encapsulated in capsules using a Dosator type capsule filling machine.

- 10 The entire scope of this invention is not limited by the specific examples described herein, but is more readily understood with reference to the appended claims.

What is claimed is:

- 15 1. Crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate.

2. The hemihydrate of claim 1 having an X-ray powder diffraction pattern comprising peaks at approximately 16, 22 and 27 degrees 2θ.

- 20 3. The hemihydrate of claim 2 wherein the pattern further comprises a peak at approximately 18 degrees 2θ.

4. The hemihydrate of claim 1 having an X-ray powder diffraction pattern comprising peaks at 15.8, 22.2 and 26.7 degrees 2θ.

- 25 5. The hemihydrate of claim 4 wherein the pattern further comprises a peak at 18.2 degrees 2θ.

6. The hemihydrate of claim 1 having an X-ray powder diffraction pattern corresponding to the representative X-ray powder diffraction patterns depicted in FIG. 6, FIG. 32, FIG. 33 and FIG. 34.

- 30 7. The hemihydrate of claim 1 having a differential scanning calorimetry thermogram comprising an endotherm with a maximum at about 268° C.

8. The hemihydrate of claim 7 wherein the thermogram further comprises an endotherm corresponding to dehydration.

9. The hemihydrate of claim 1 having a differential scanning calorimetry thermogram corresponding to the representative differential scanning calorimetry thermograms depicted in FIG. 9, FIG. 42, FIG. 43 and FIG. 44.

- 40 10. The hemihydrate of claim 1 having between approximately 0.46 and approximately 0.59 moles of water per mole of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

45 11. The hemihydrate of claim 1 having a thermogravimetric analysis thermogram comprising a weight loss of between about 3.1% and about 4.0% when heated from about 30° C. to about 175° C.

12. The hemihydrate of claim 1 having an infrared spectrum comprising peaks at approximately 3513 and 1960 cm<sup>-1</sup>.

13. The hemihydrate of claim 1 having an infrared spectrum corresponding to the representative infrared spectrum depicted in FIG. 7.

- 55 14. The hemihydrate of claim 1 having a Raman spectrum corresponding to the representative Raman spectrum depicted in FIG. 8.

\* \* \* \* \*

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 7,465,800 B2  
APPLICATION NO. : 10/934863  
DATED : December 16, 2008  
INVENTOR(S) : Jaworsky et al.

Page 1 of 11

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Please replace Drawing Sheets 15, 26, 31, 32, 33, 35, 36, 38, 39, and 40 (of 48) with the Replacement Drawing Sheets on the following pages of this Certificate of Correction:

Page 2 of 11: Replacement Drawing Sheet 15 of 48 (Fig. 15);

Page 3 of 11: Replacement Drawing Sheet 26 of 48 (Fig. 26);

Page 4 of 11: Replacement Drawing Sheet 31 of 48 (Fig. 31);

Page 5 of 11: Replacement Drawing Sheet 32 of 48 (Figs. 32 and 33);

Page 6 of 11: Replacement Drawing Sheet 33 of 48 (Figs. 34 and 35);

Page 7 of 11: Replacement Drawing Sheet 35 of 48 (Figs. 37 and 38);

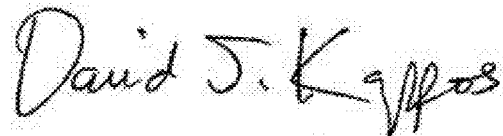
Page 8 of 11: Replacement Drawing Sheet 36 of 48 (Figs. 39 and 40);

Page 9 of 11: Replacement Drawing Sheet 38 of 48 (Figs. 42 and 43);

Page 10 of 11: Replacement Drawing Sheet 39 of 48 (Figs. 44 and 45);

Page 11 of 11: Replacement Drawing Sheet 40 of 48 (Fig. 46).

Signed and Sealed this  
Nineteenth Day of April, 2011

A handwritten signature in black ink that reads "David J. Kappos". The signature is written in a cursive, slightly slanted style.

David J. Kappos  
*Director of the United States Patent and Trademark Office*



## CERTIFICATE OF CORRECTION (continued)

Page 2 of 11

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Sheet 15 of 48

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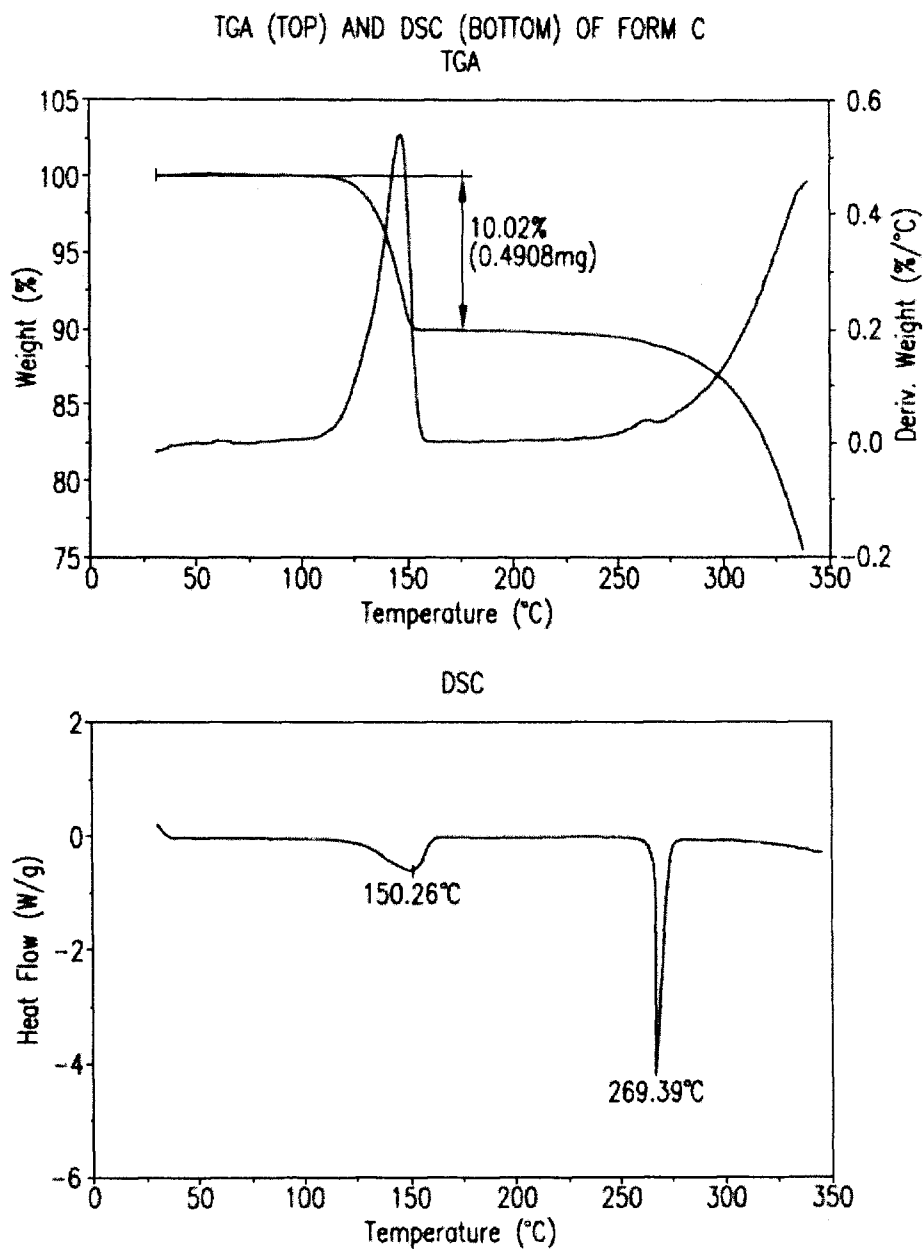


FIG.15

CERTIFICATE OF CORRECTION (continued)

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Sheet 26 of 48

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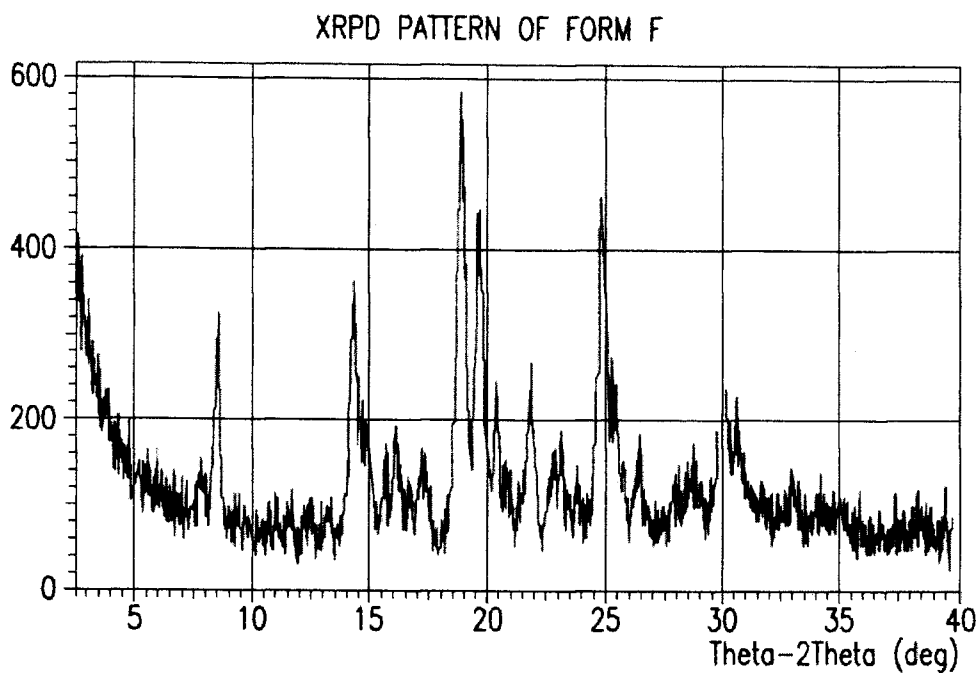


FIG.26

## CERTIFICATE OF CORRECTION (continued)

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U.S. Patent

Dec. 16, 2008

Sheet 31 of 48

7,465,800 B2

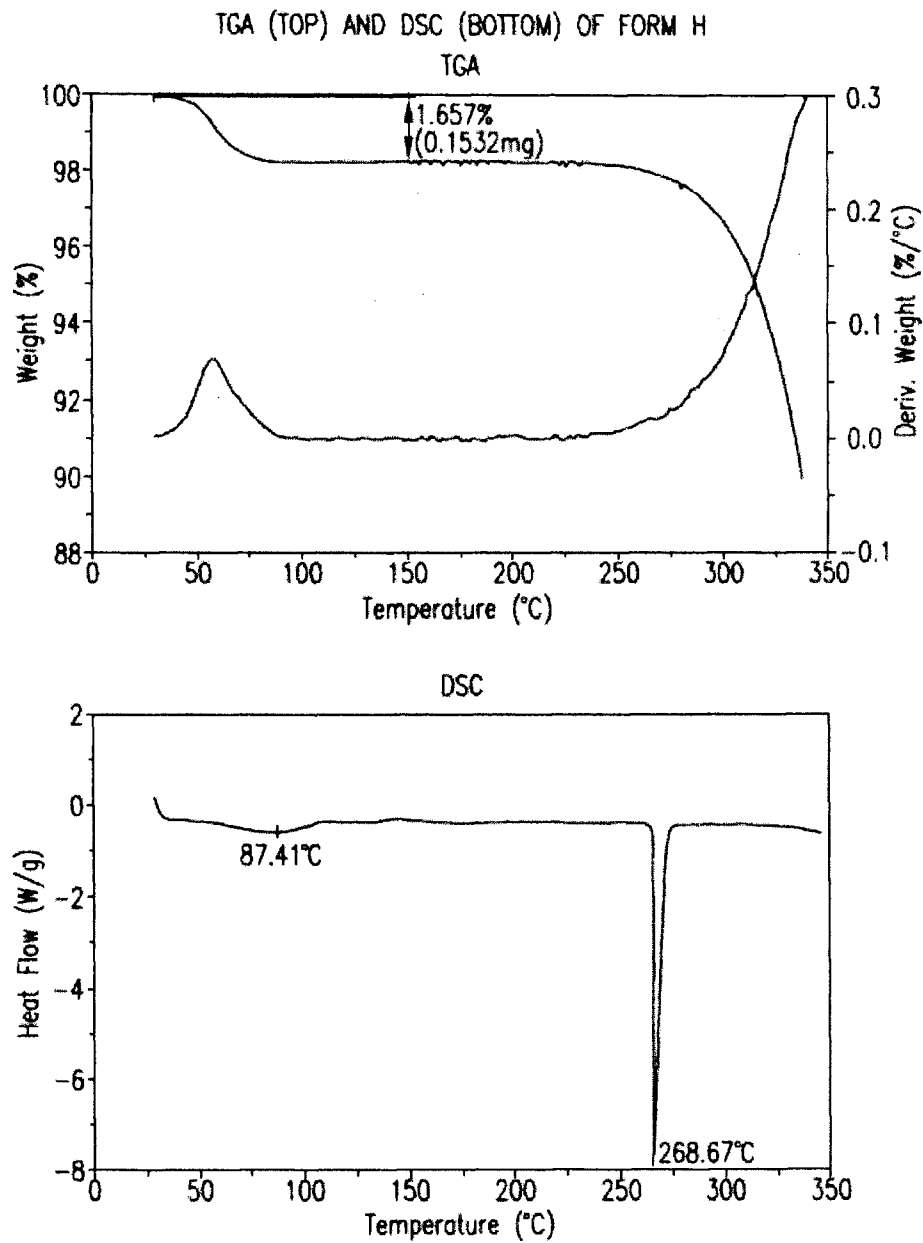


FIG.31

## CERTIFICATE OF CORRECTION (continued)

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## XRPD PATTERN OF POLYMORPH B

File: Process 5274-104-B

Date: 06-04-04 16:10 Step: 0.020° Cnt Time: 0.500 Sec.

Range: 1.50-40.00 (Deg) Step Scan Rate: 2.40 Deg/min.

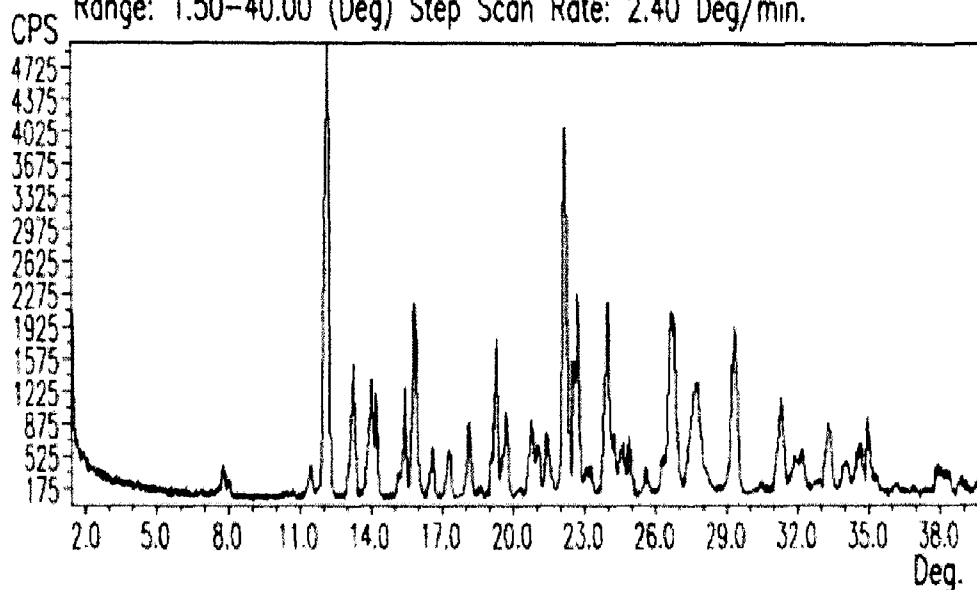


FIG.32

## XRPD PATTERN OF POLYMORPH B

File: Process 5274-100-C

Date: 06-02-04 16:11 Step: 0.020° Cnt Time: 0.500 Sec.

Range: 1.50-40.00 (Deg) Step Scan Rate: 2.40 Deg/min.

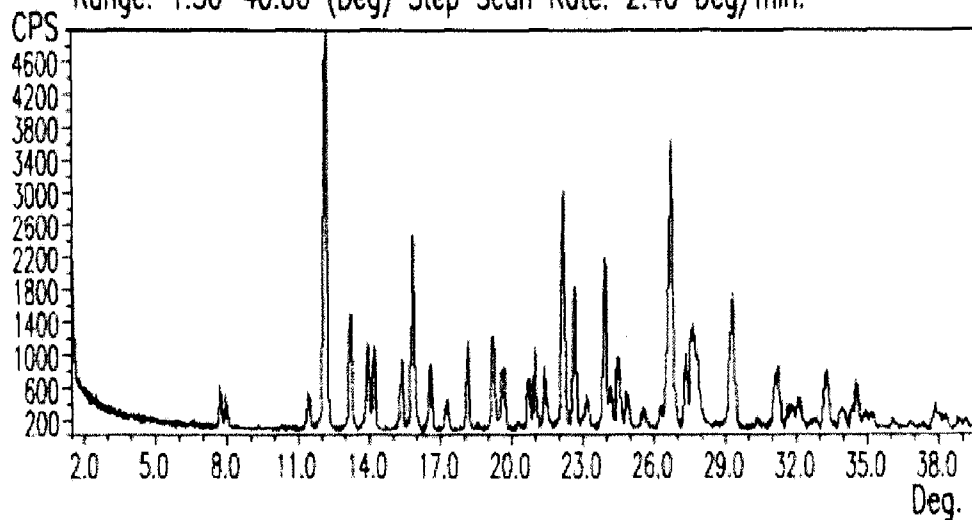


FIG.33

## CERTIFICATE OF CORRECTION (continued)

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## XRPD PATTERN OF POLYMORPH B

File: Process 5222-157-C

Date: 06/04/04 15:07 Step: 0.020° Cnt Time: 0.500 Sec.

Range: 1.50-40.00 (Deg) Step Scan Rate: 2.40 Deg/min.

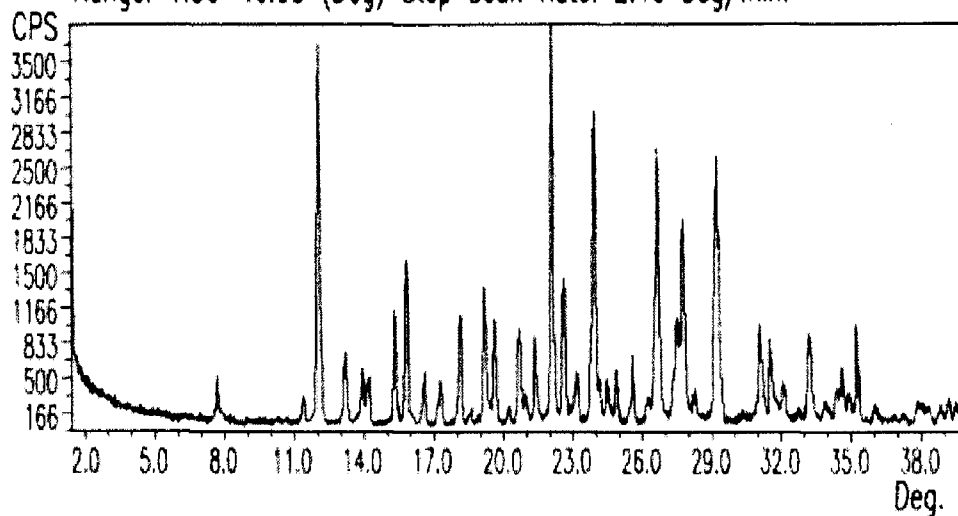


FIG.34

## XRPD PATTERN OF POLYMORPH E

File: Process 5222-152-B Form E

Date: 05/21/04 10:46 Step: 0.020° Cnt Time: 0.500 Sec.

Range: 1.50-40.00 (Deg) Step Scan Rate: 2.40 Deg/min.

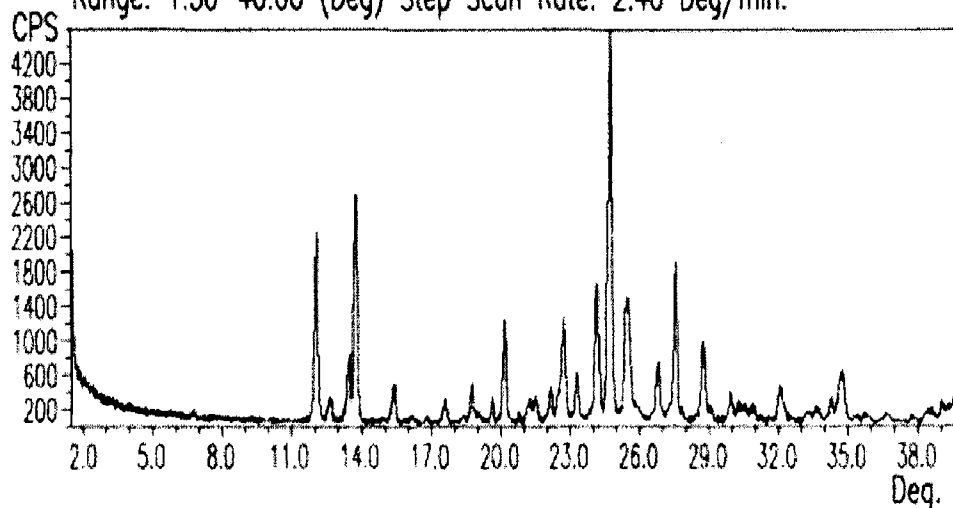


FIG.35

## CERTIFICATE OF CORRECTION (continued)

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TGA CURVE OF POLYMORPH B  
Sample: 5274-104-B  
Size 7.8320mg  
Method: Ramp  
TGA  
Run Date: 09-Jun-04 17:04  
Instrument: TGA 0.500 V5.3 Build 171

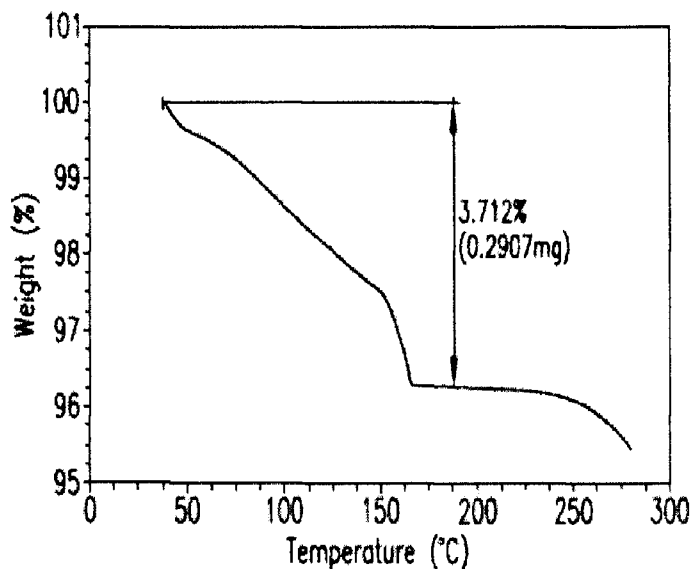


FIG.37

TGA CURVE OF POLYMORPH B  
Sample: 5274-100-C  
Size 10.9430mg  
Method: Ramp  
Comment: Evotec Batch 1675C H2O  
TGA  
Run Date: 03-Jun-04 11:20  
Instrument: TGA 0.500 V5.3 Build 171

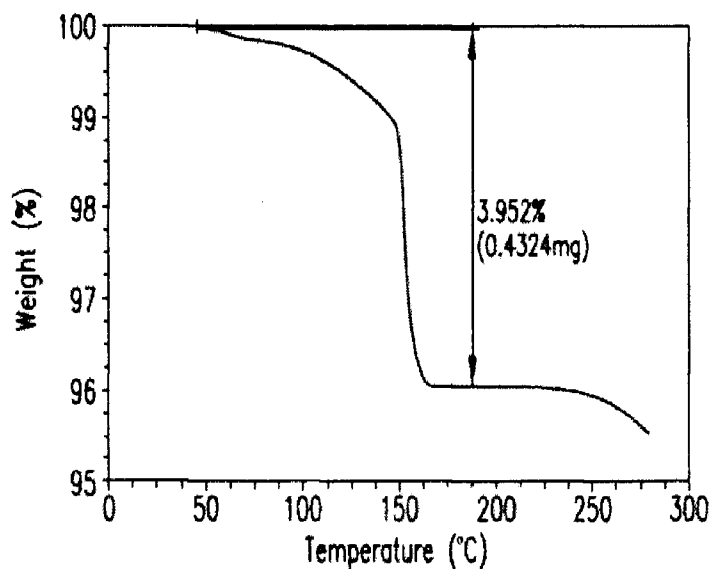


FIG.38

## CERTIFICATE OF CORRECTION (continued)

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TGA CURVE OF POLYMORPH B  
TGA  
Sample: 5222-157-C  
Size: 24.4610mg  
Method: Ramp  
Comment: Evotec Batch 17  
Run Date: 07-Jun-04 09:46  
Instrument: TGA 0.500 V5.3 Build 171

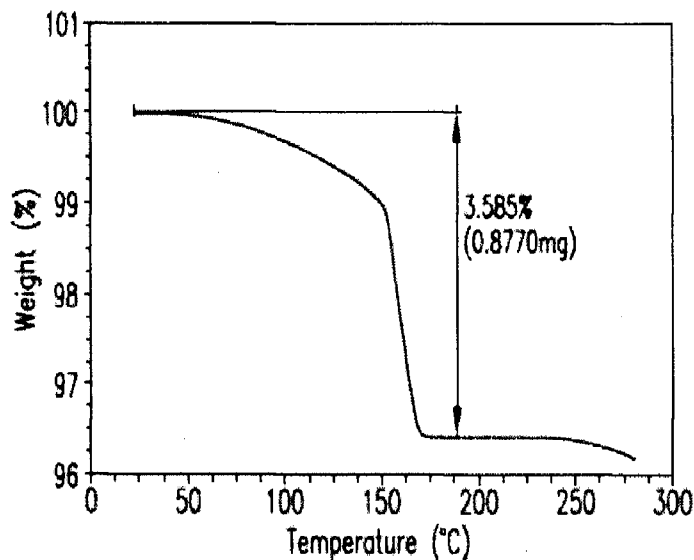


FIG.39

TGA CURVE OF POLYMORPH E  
TGA  
Sample: 5222-152-B  
Size: 11.3850mg  
Method: Ramp  
Comment: Form E  
Run Date: 21-May-04 09:34  
Instrument: TGA 0.500 V5.3 Build 171

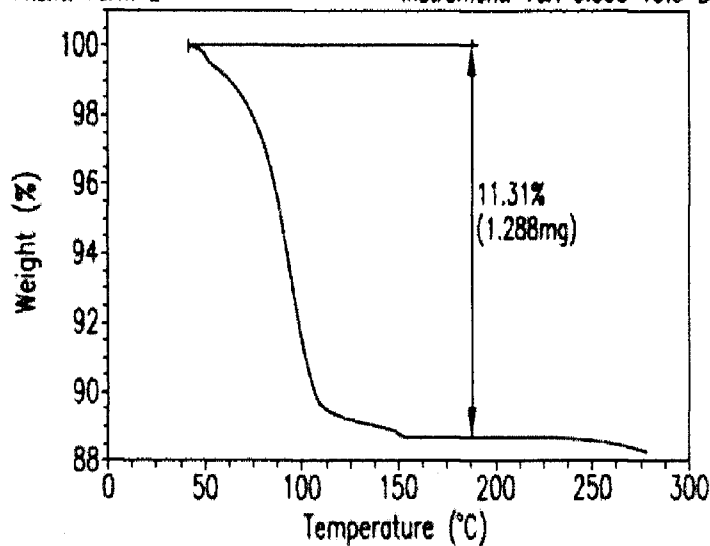


FIG.40

## CERTIFICATE OF CORRECTION (continued)

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DSC OF POLYMORPH B  
DSC  
Sample: 5274-104-B  
Size: 0.0000mg  
Method: Cell constant calibration  
Run Date: 07-Jun-04 11:30  
Comment: QAI batch 15 22b  
Instrument: DSC Q 1000 V7.3 Build 249

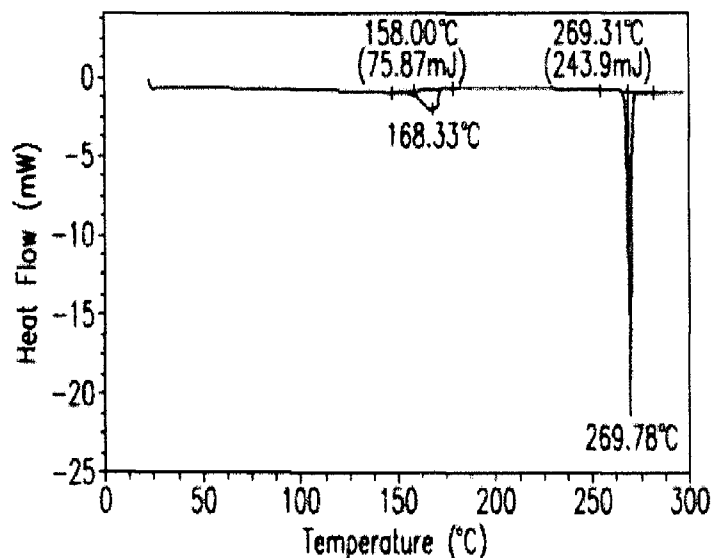


FIG.42

DSC OF POLYMORPH B  
DSC  
Sample: 5274-100-C  
Size: 0.0000mg  
Method: Cell constant calibration  
Run Date: 02-Jun-04 17:01  
Comment: Evotec Batch 1675C 10vol H2O 24h 3h dry  
Instrument: DSC Q 1000 V7.3 Build 249

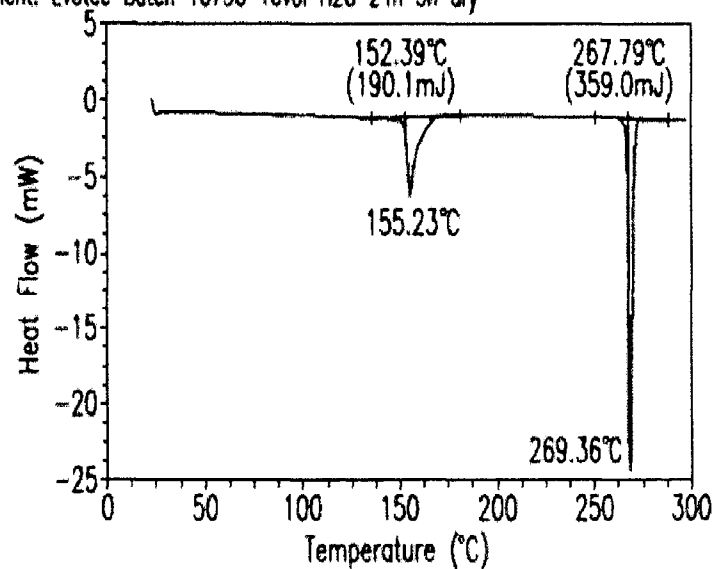


FIG.43



## CERTIFICATE OF CORRECTION (continued)

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Dec. 16, 2008

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DSC OF POLYMORPH B  
DSC  
Sample: 5222-157-C  
Size: 0.0000mg  
Method: Cell constant calibration  
Run Date: 07-Jun-04 09:45  
Comment: OAI batch 17  
Instrument: DSC Q 1000 V7.3 Build 249

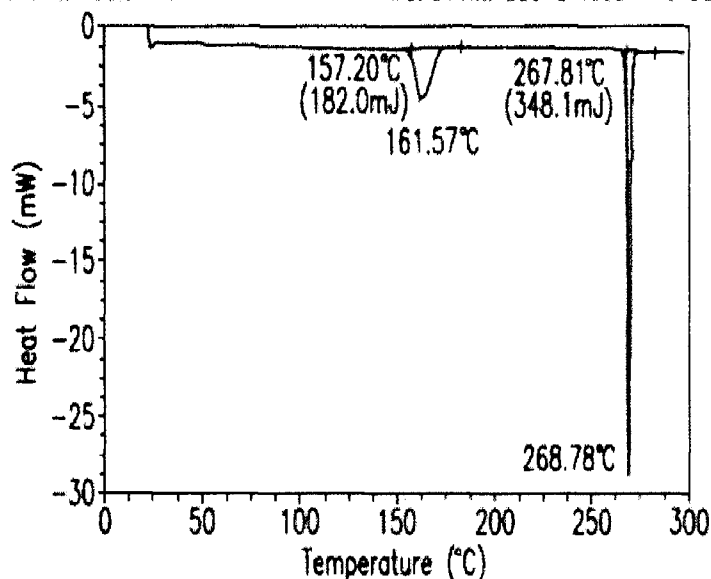


FIG.44

DSC OF POLYMORPH E  
DSC  
Sample: 5222-152-B  
Size: 3.4500mg  
Method: Cell constant calibration  
Run Date: 21-May-04 09:32  
Comment: Evotec batch 15 Form E  
Instrument: DSC Q 1000 V7.3 Build 249

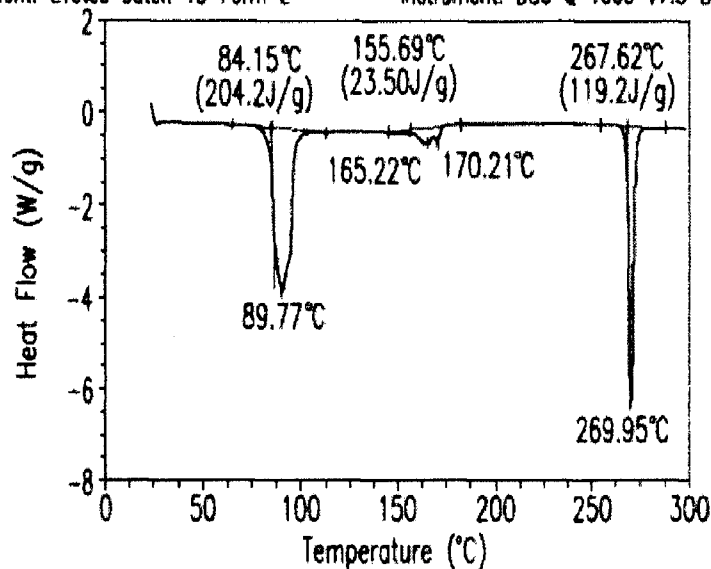


FIG.45

## CERTIFICATE OF CORRECTION (continued)

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U.S. Patent

Dec. 16, 2008

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## DSC OF POLYMORPH MIXTURE

Sample: 5222-161-A

DSC

Size: 0.0000mg

Method: Cell constant calibration

Run Date: 11-Jun-04 12:44

Comment: OAI batch 15

Instrument: DSC Q 1000 V7.3 Build 249

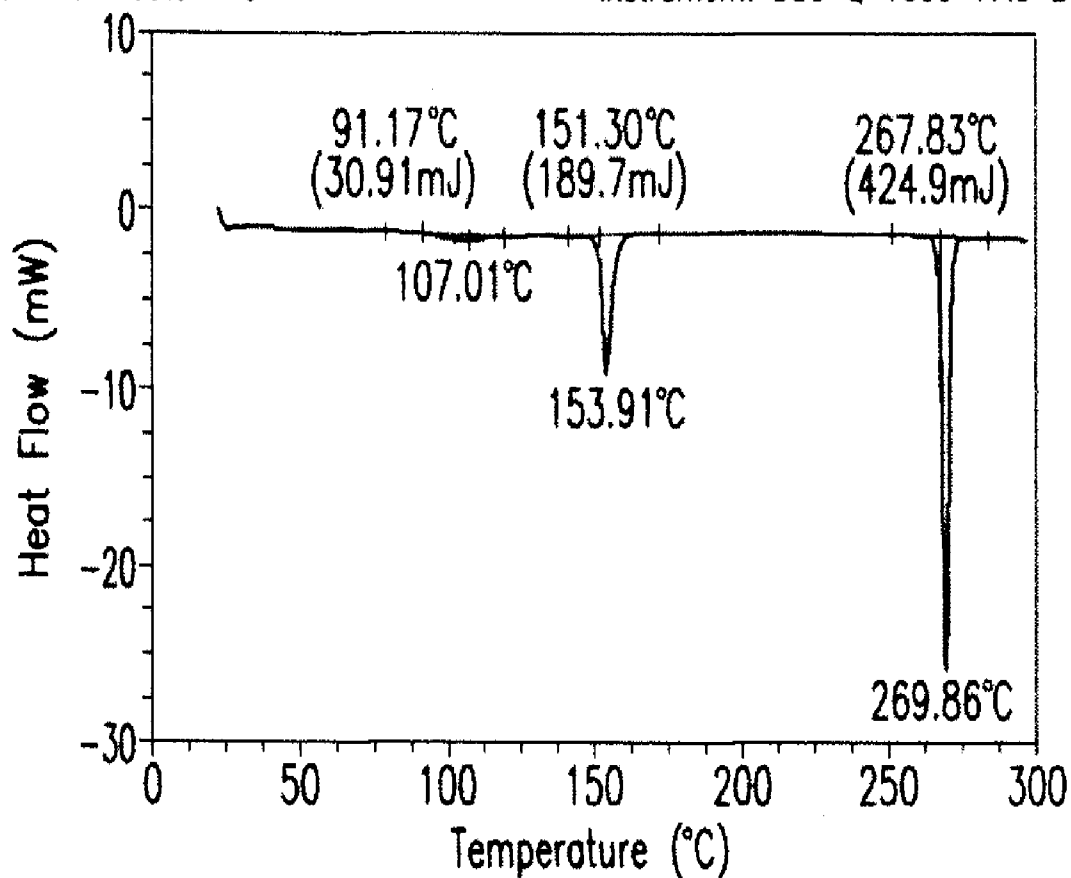


FIG.46

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 7,465,800 B2  
APPLICATION NO. : 10/934863  
DATED : December 16, 2008  
INVENTOR(S) : Jaworsky et al.

Page 1 of 1

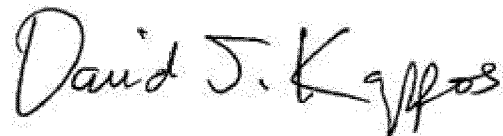
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

The first or sole Notice should read --

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b)  
by 966 days.

Signed and Sealed this  
Fifteenth Day of May, 2012

A handwritten signature in black ink that reads "David J. Kappos". The signature is written in a cursive, flowing style.

David J. Kappos  
*Director of the United States Patent and Trademark Office*

# EXHIBIT C



US007855217B2

(12) **United States Patent**  
**Jaworsky et al.**

(10) **Patent No.:** **US 7,855,217 B2**  
(45) **Date of Patent:** **\*Dec. 21, 2010**

(54) **POLYMORPHIC FORMS OF  
3-(4-AMINO-1-OXO-1,3  
DIHYDRO-ISOINDOL-2-YL)-  
PIPERIDINE-2,6-DIONE**

(75) Inventors: **Markian S. Jaworsky**, Hopewell, NJ  
(US); **Roger Shen-Chu Chen**, Edison,  
NJ (US); **George W. Muller**,  
Bridgewater, NJ (US)

(73) Assignee: **Celgene Corporation**, Summit, NJ (US)

(\*) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 82 days.

This patent is subject to a terminal dis-  
claimer.

(21) Appl. No.: **12/335,395**

(22) Filed: **Dec. 15, 2008**

(65) **Prior Publication Data**

US 2009/0149500 A1 Jun. 11, 2009

**Related U.S. Application Data**

(62) Division of application No. 10/934,863, filed on Sep.  
3, 2004, now Pat. No. 7,465,800.

(60) Provisional application No. 60/499,723, filed on Sep.  
4, 2003.

(51) **Int. Cl.**

**A61K 31/454** (2006.01)

**C07D 401/04** (2006.01)

(52) **U.S. Cl.** ..... **514/323**; 546/200

(58) **Field of Classification Search** ..... 514/323;  
546/200

See application file for complete search history.

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*Primary Examiner*—Celia Chang  
(74) *Attorney, Agent, or Firm*—Jones Day

(57) **ABSTRACT**

Polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoin-  
dol-2-yl)-piperidine-2,6-dione are disclosed. Compositions  
comprising the polymorphic forms, methods of making the  
polymorphic forms and methods of their use are also dis-  
closed.

**10 Claims, 48 Drawing Sheets**

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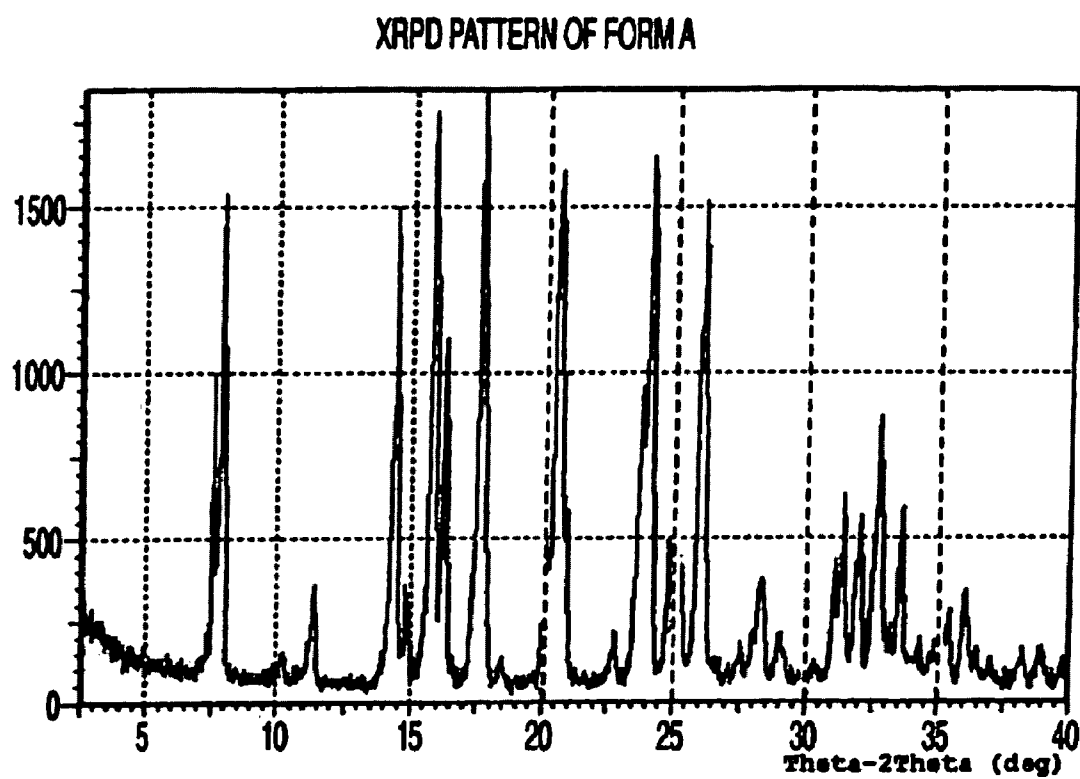
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*Fig. 1*

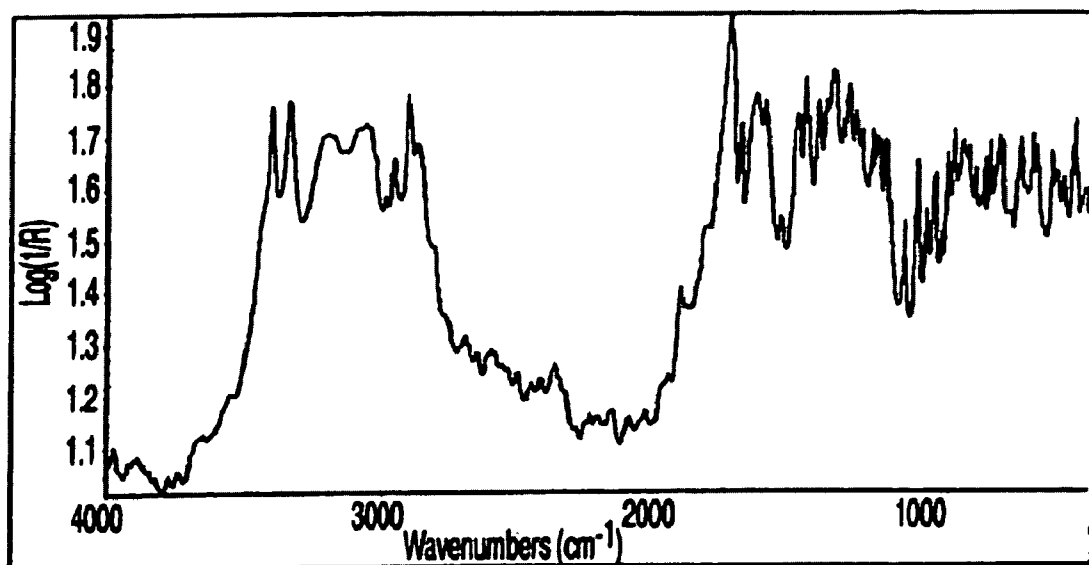
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IR SPECTRUM OF FORM A



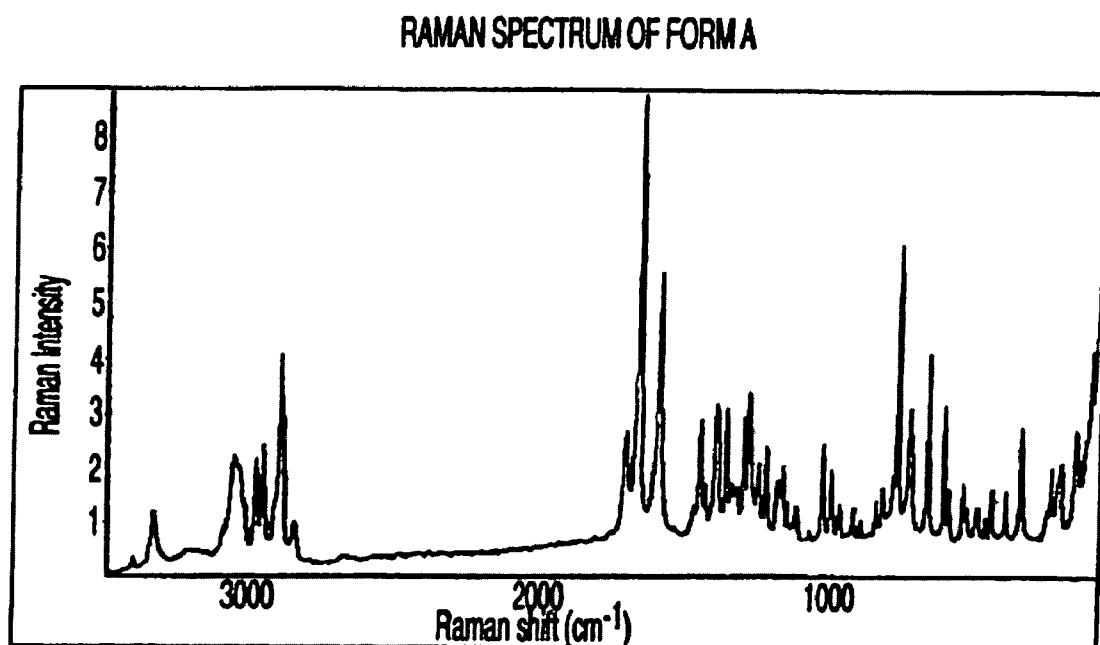
*Fig. 2*

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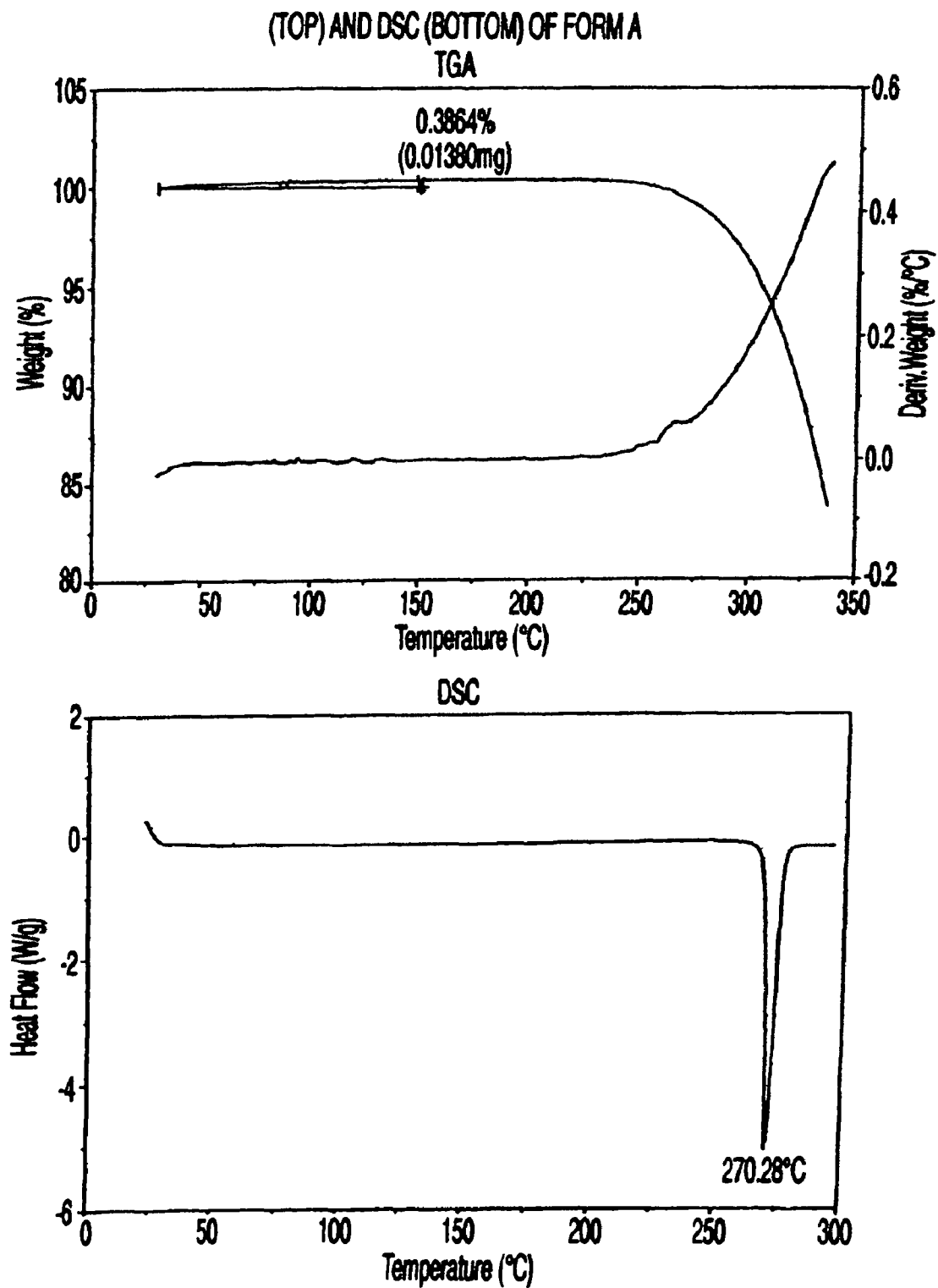
*Fig. 3*

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*Fig. 4*

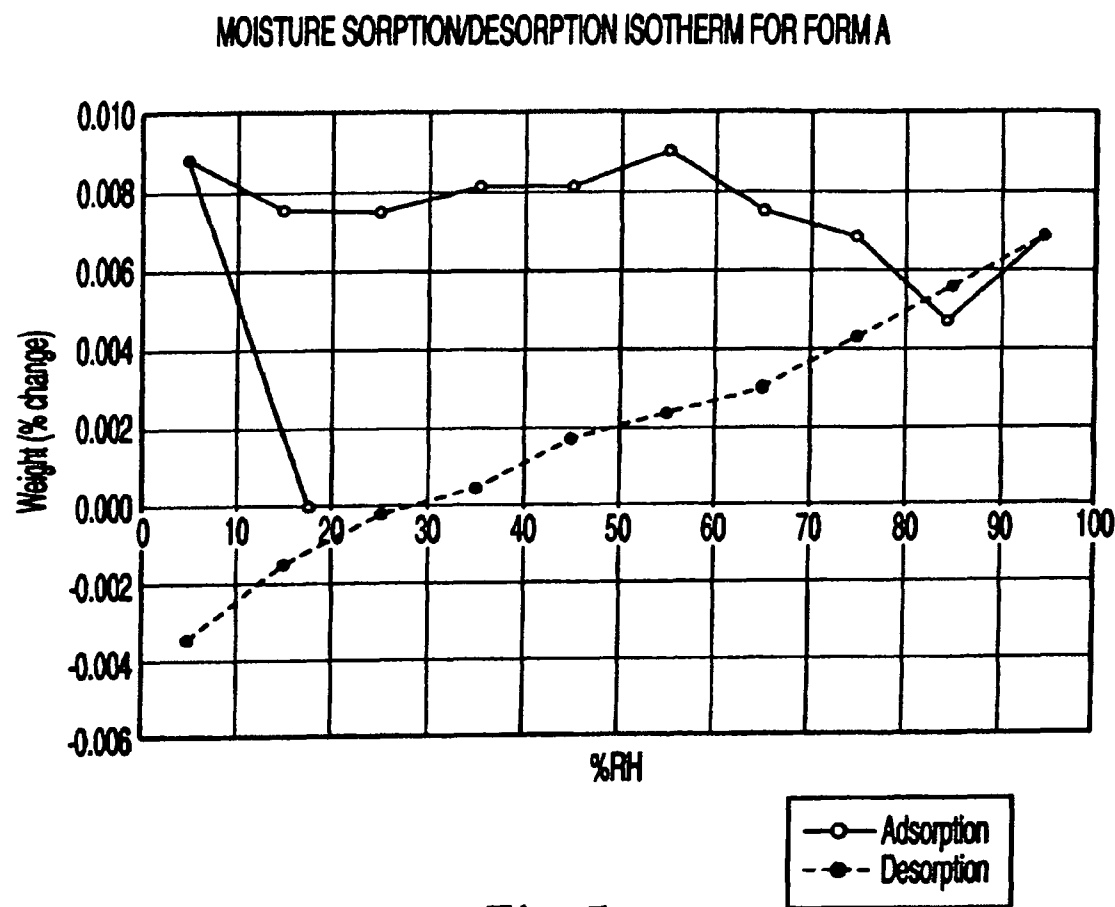


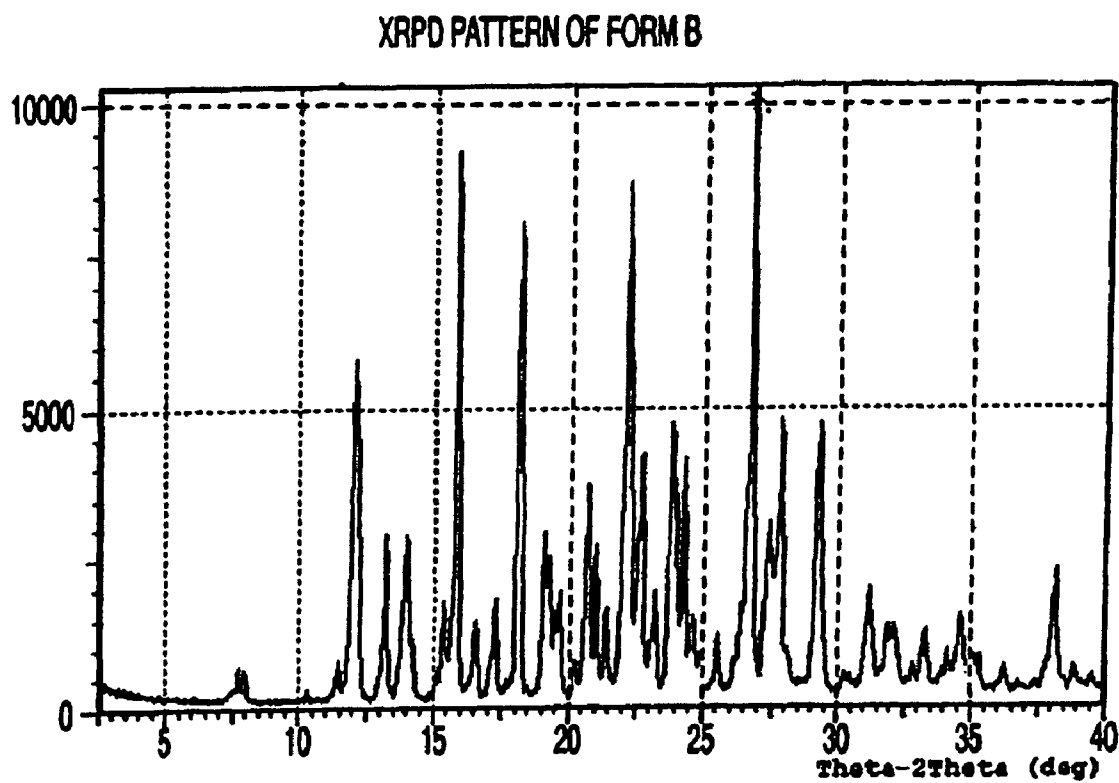
Fig. 5

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*Fig. 6*

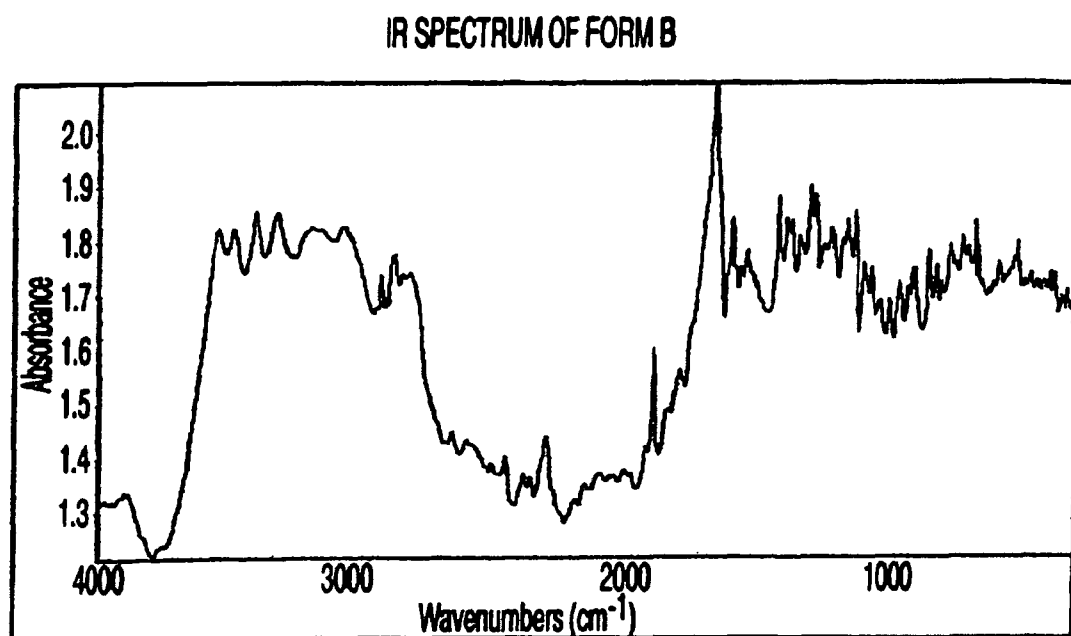


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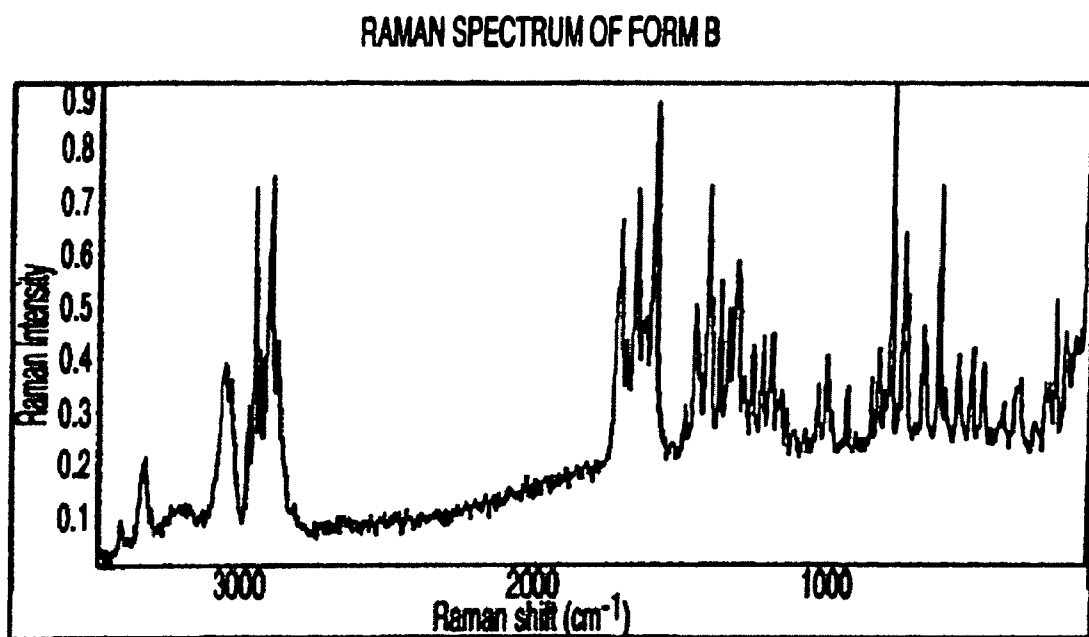
*Fig. 7*

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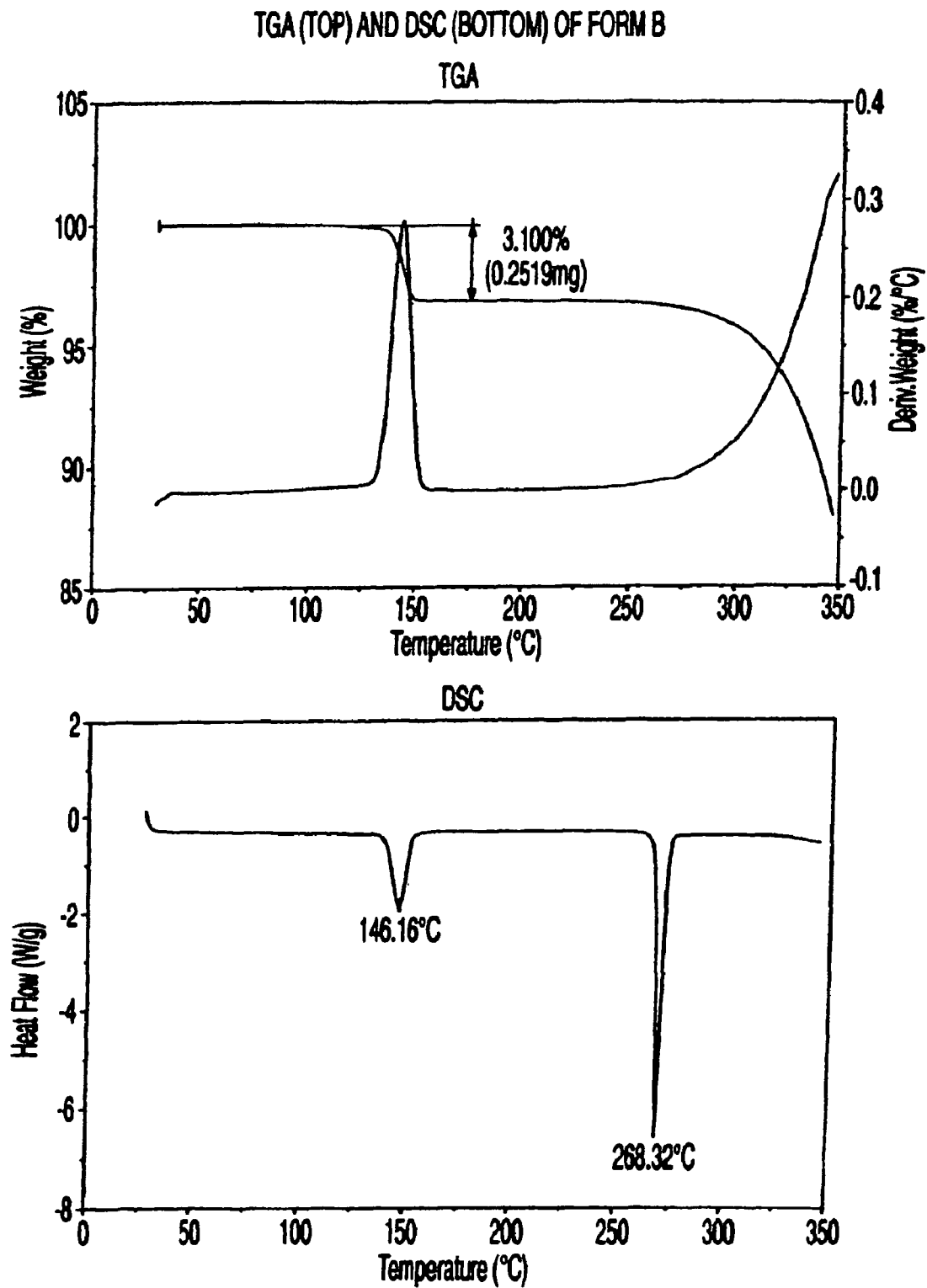
*Fig. 8*

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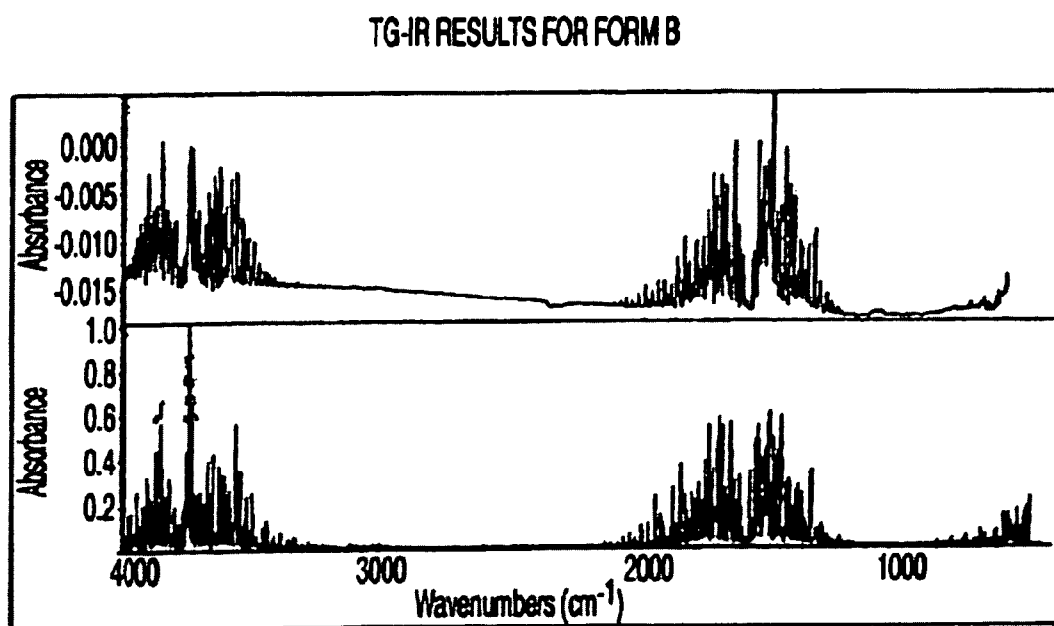
*Fig. 9*

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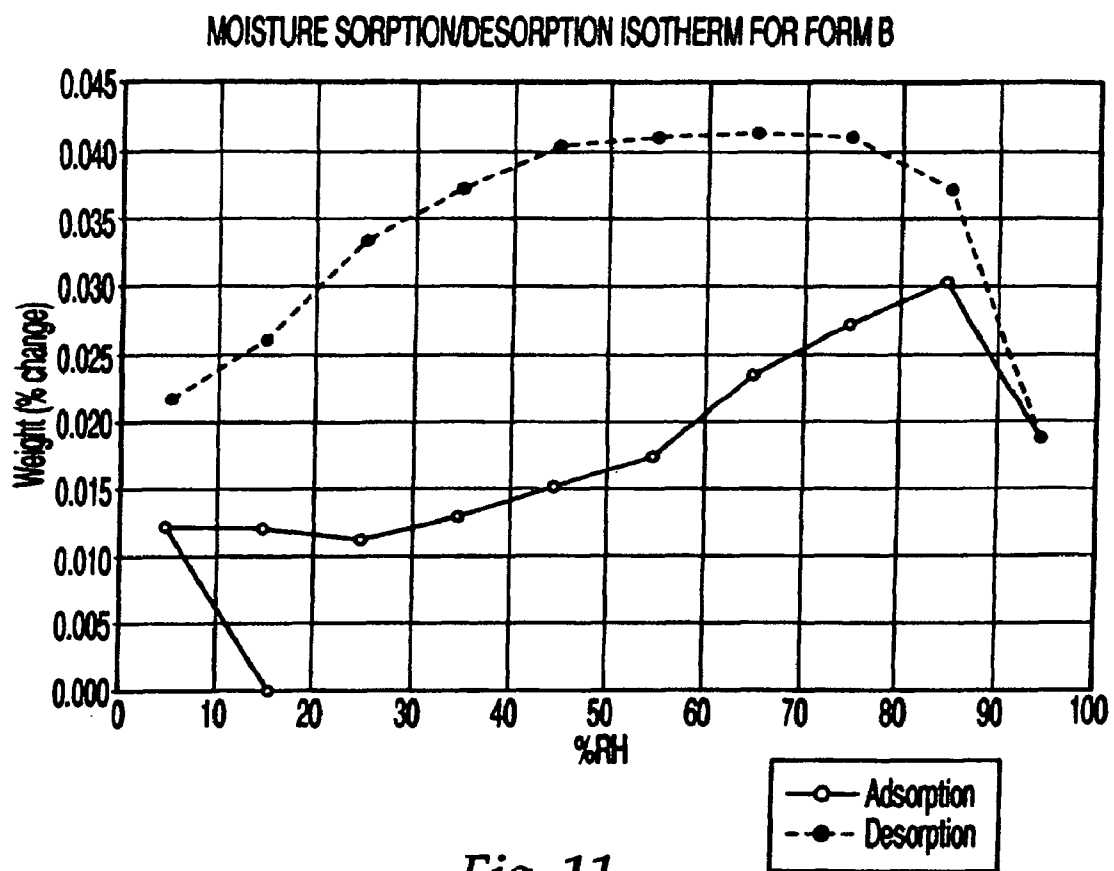
*Fig. 10*

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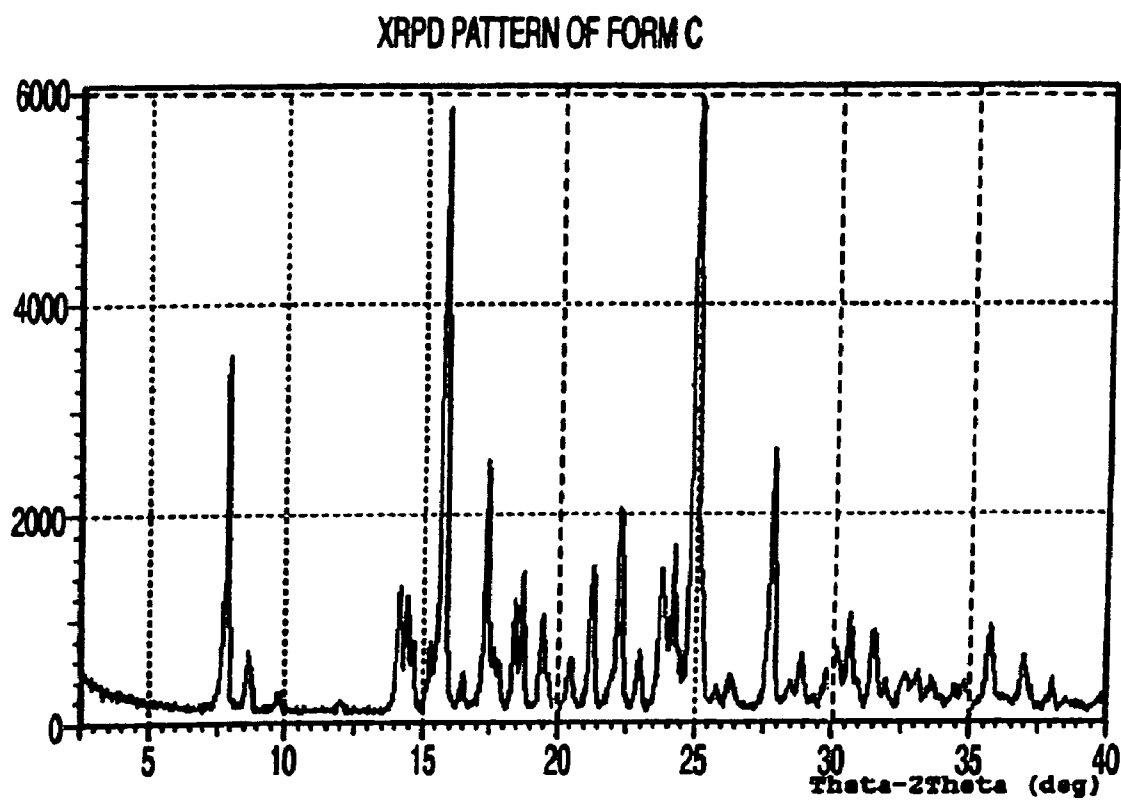


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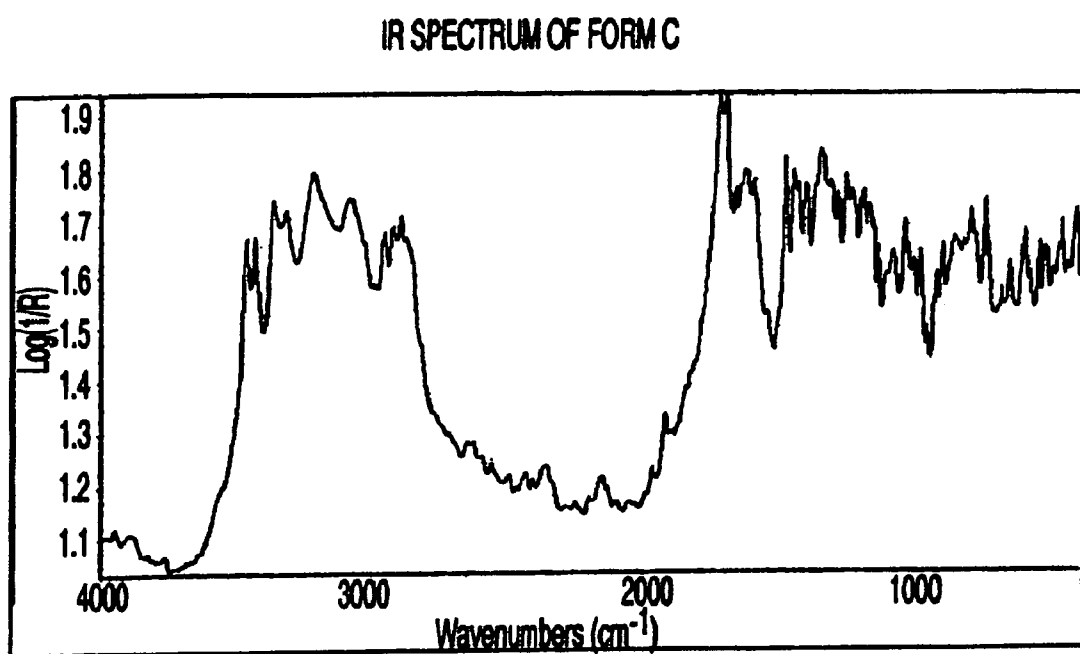
*Fig. 12*

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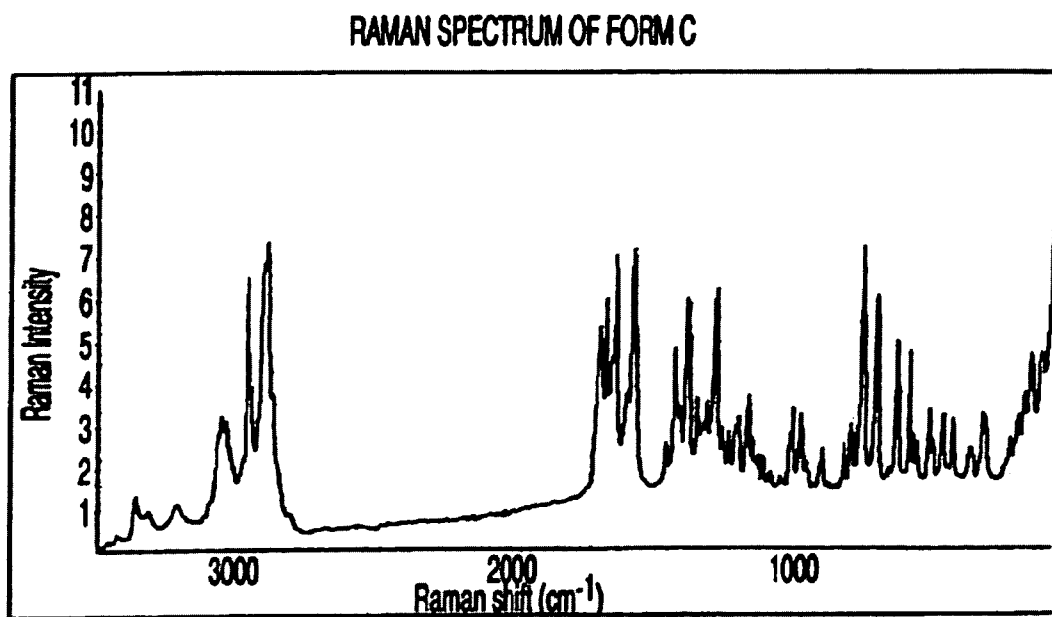
*Fig. 13*

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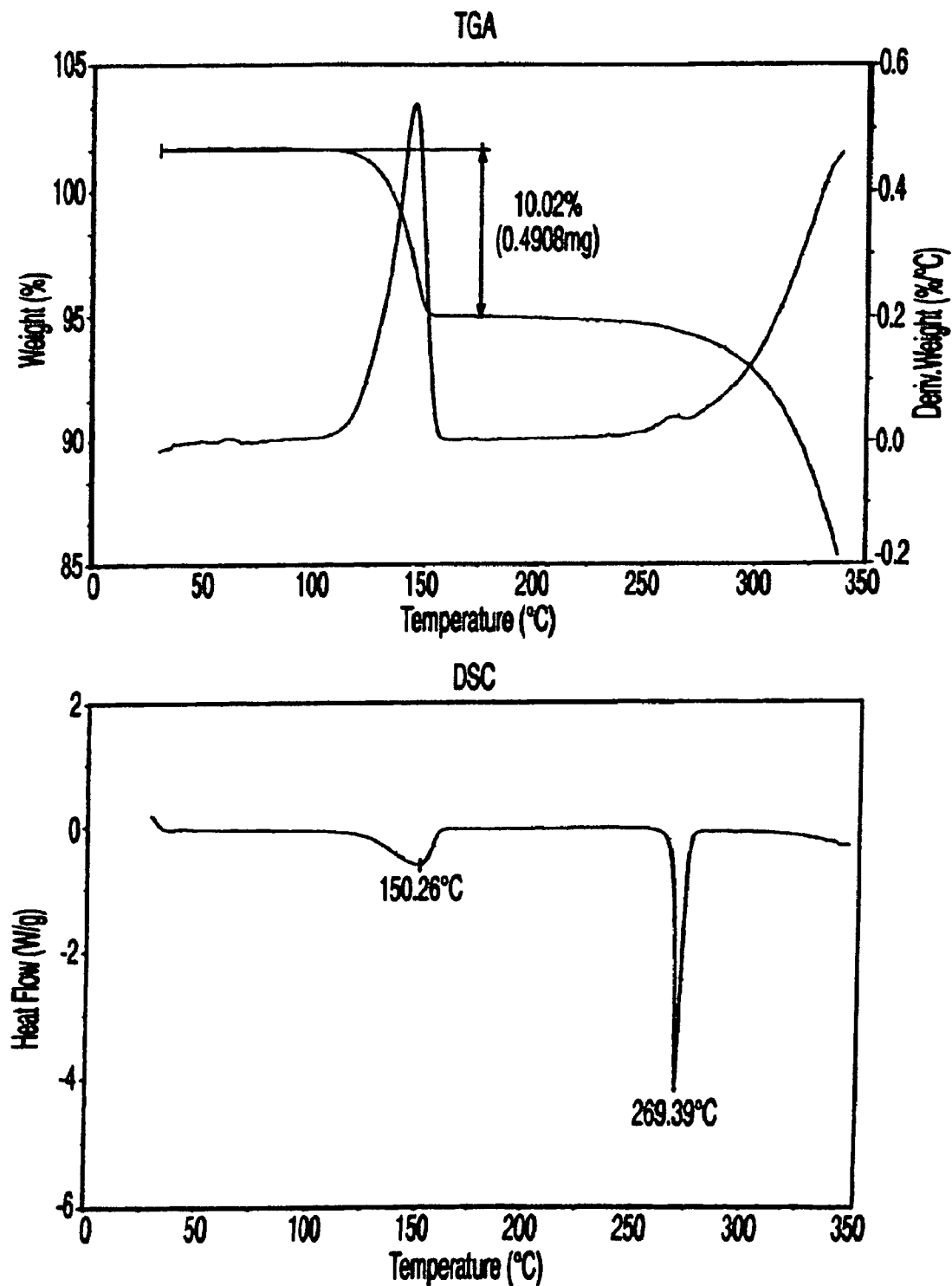
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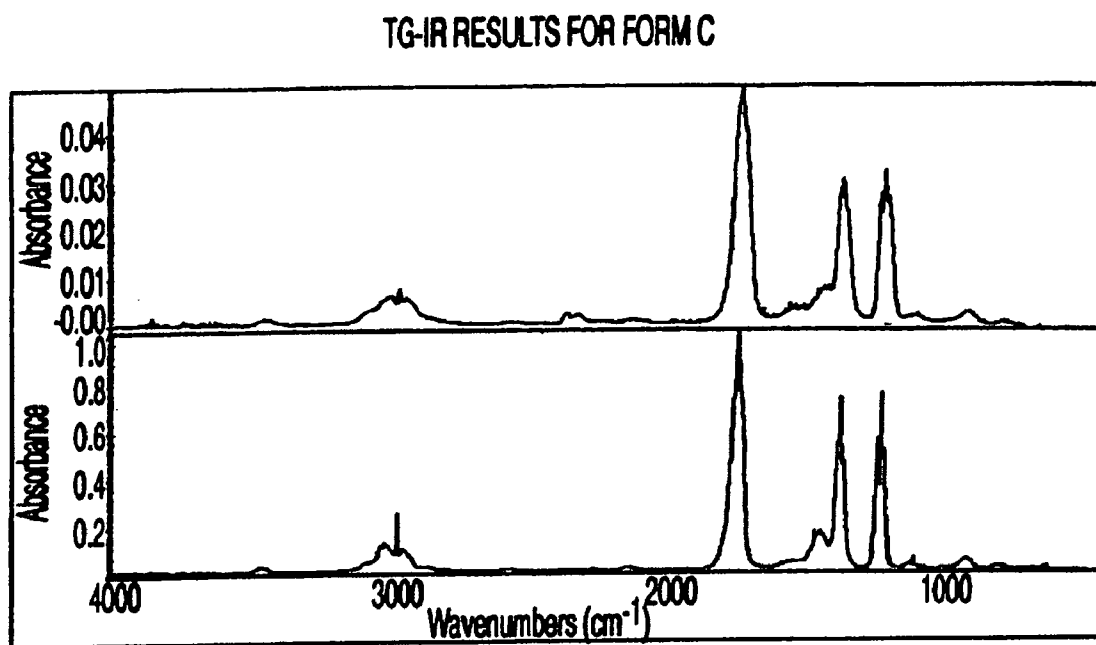


*Fig. 14*



## TGA (TOP) AND DSC (BOTTOM) OF FORM C

*Fig. 15*



*Fig. 16*

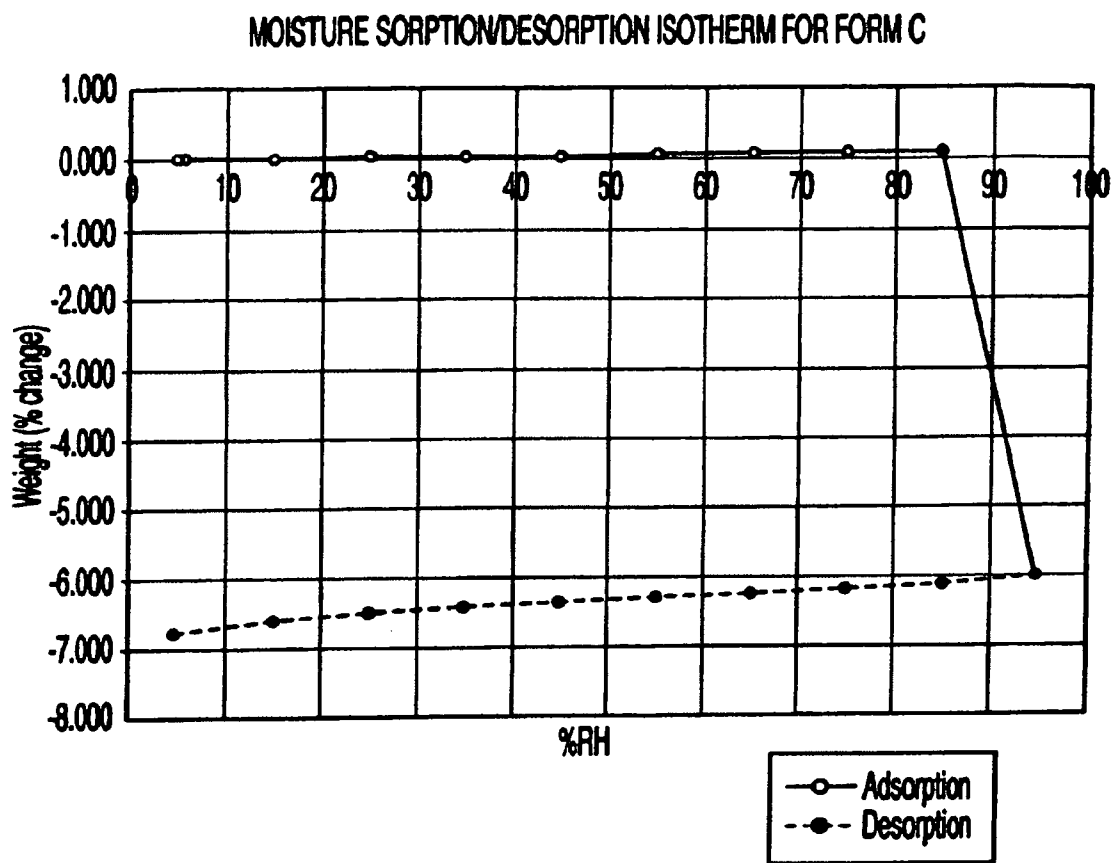


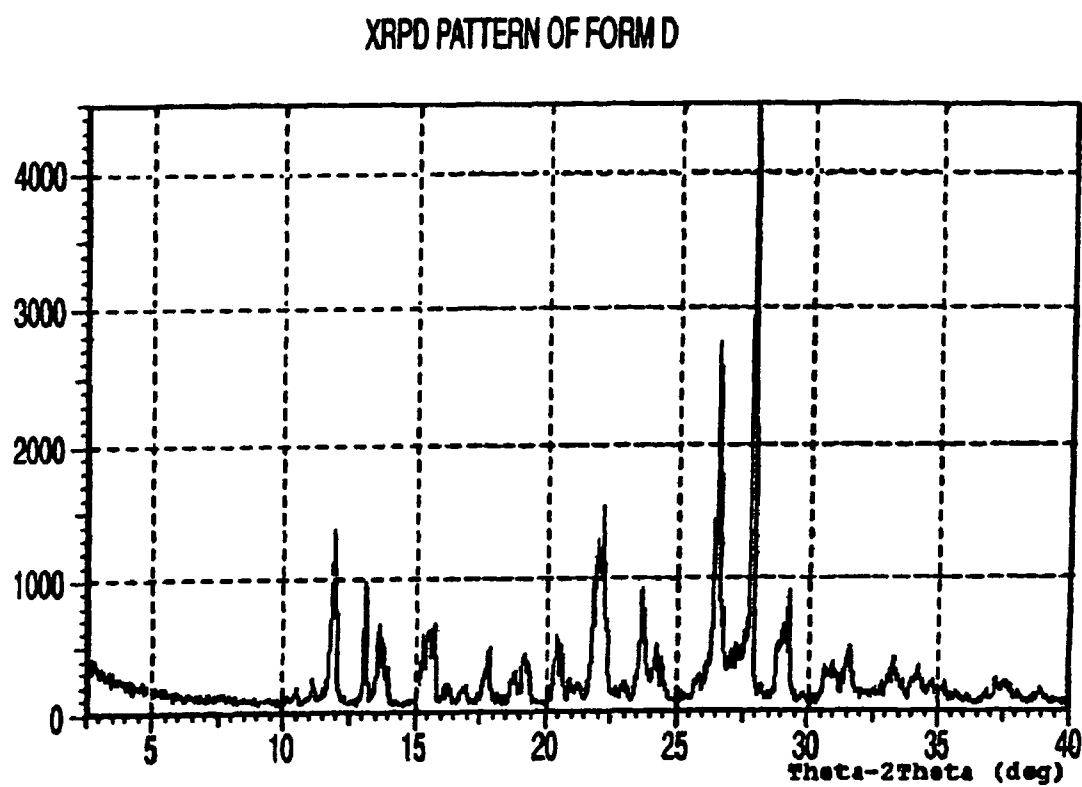
Fig. 17

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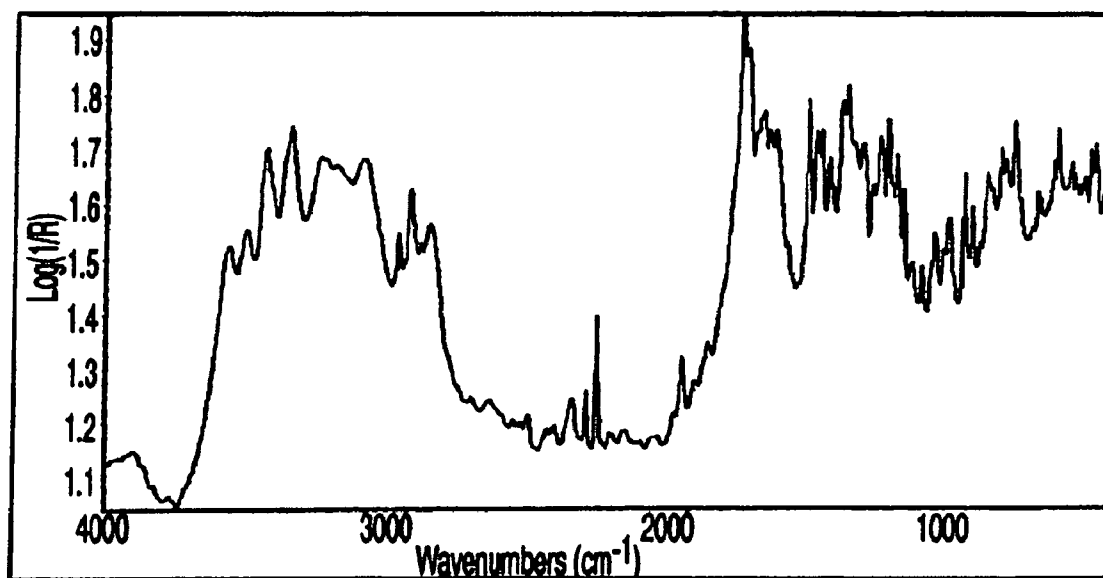
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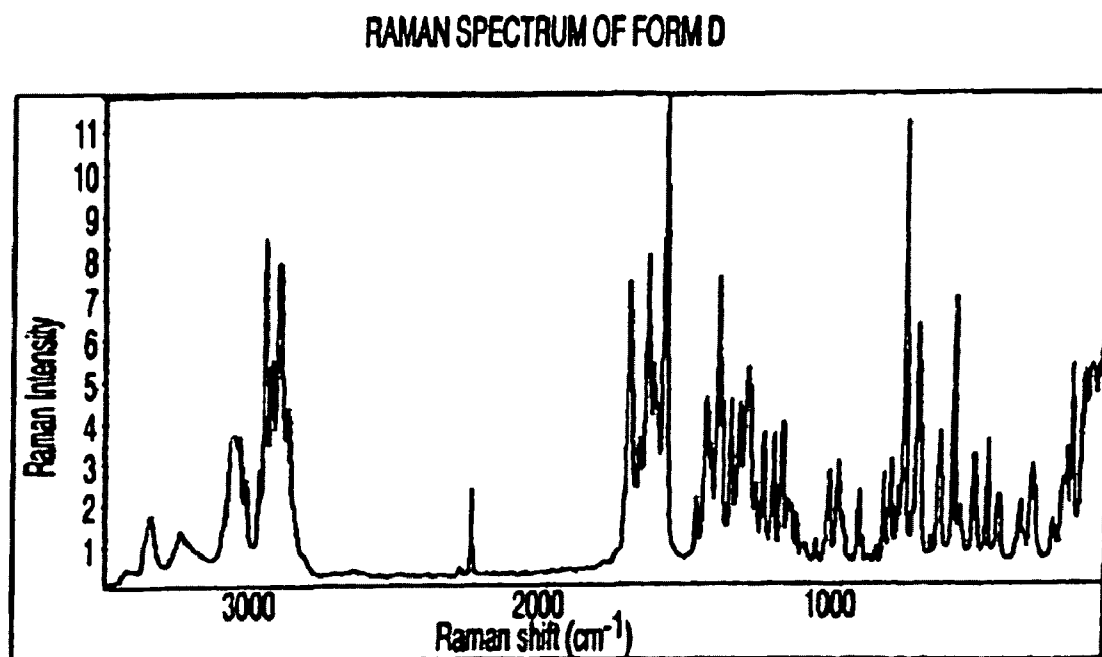


*Fig. 18*

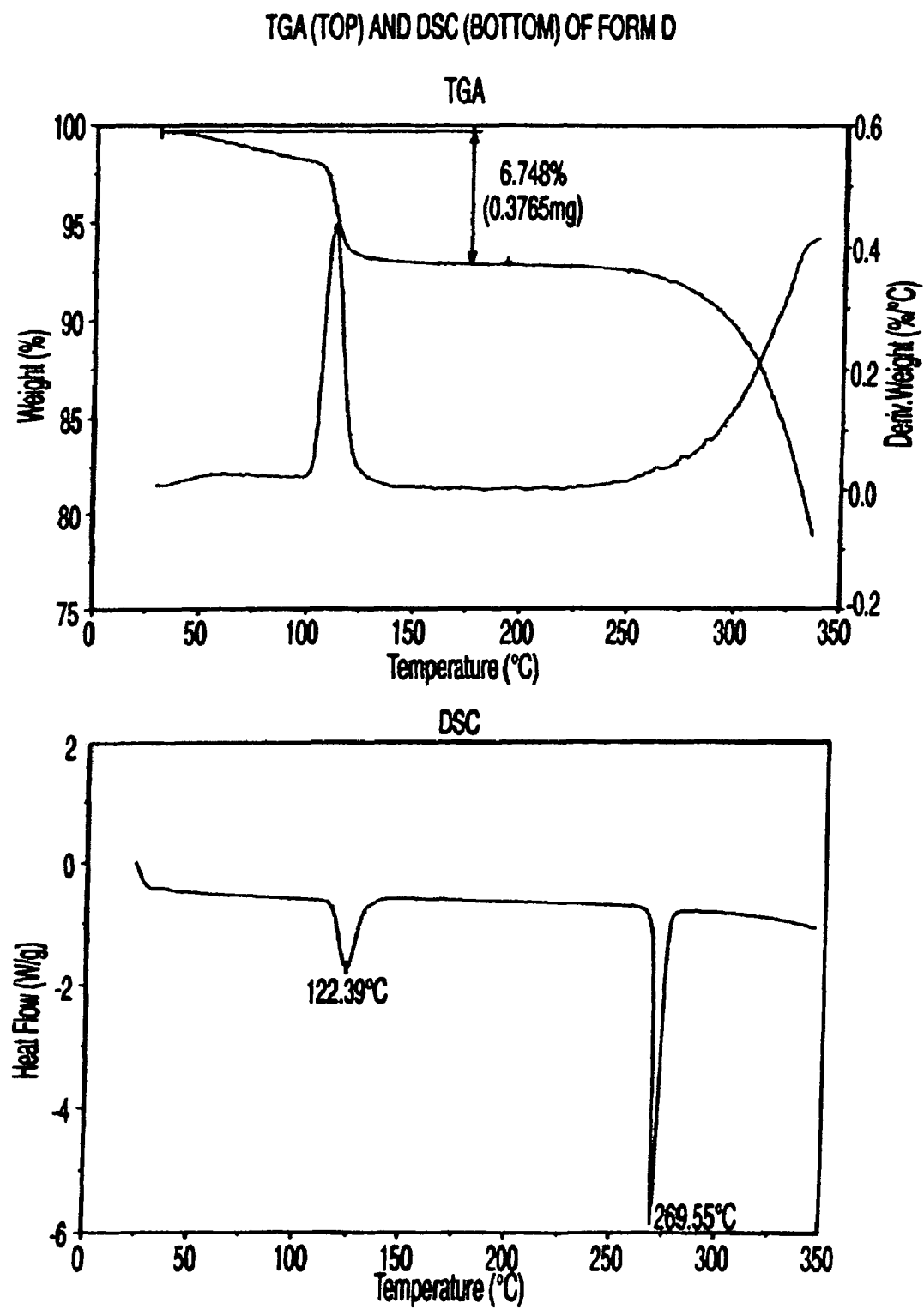
IR SPECTRUM OF FORM D

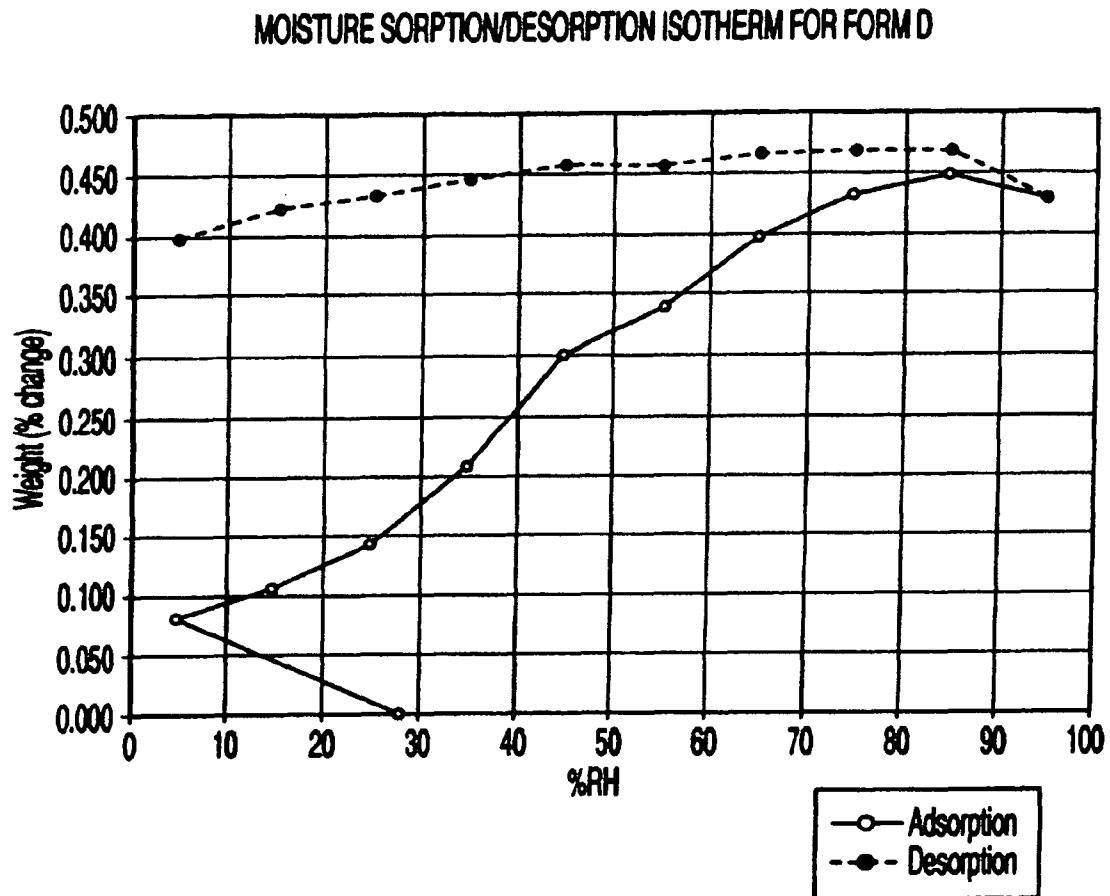


*Fig. 19*



*Fig. 20*

*Fig. 21*

*Fig. 22*

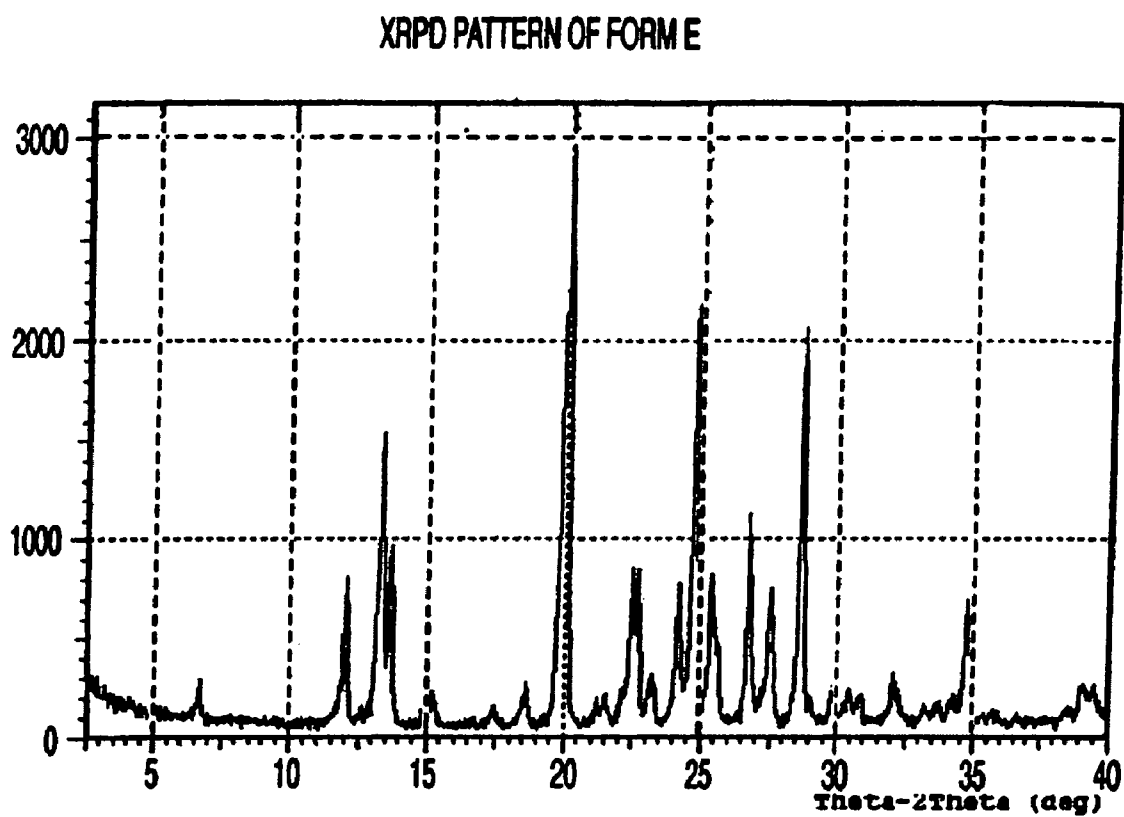


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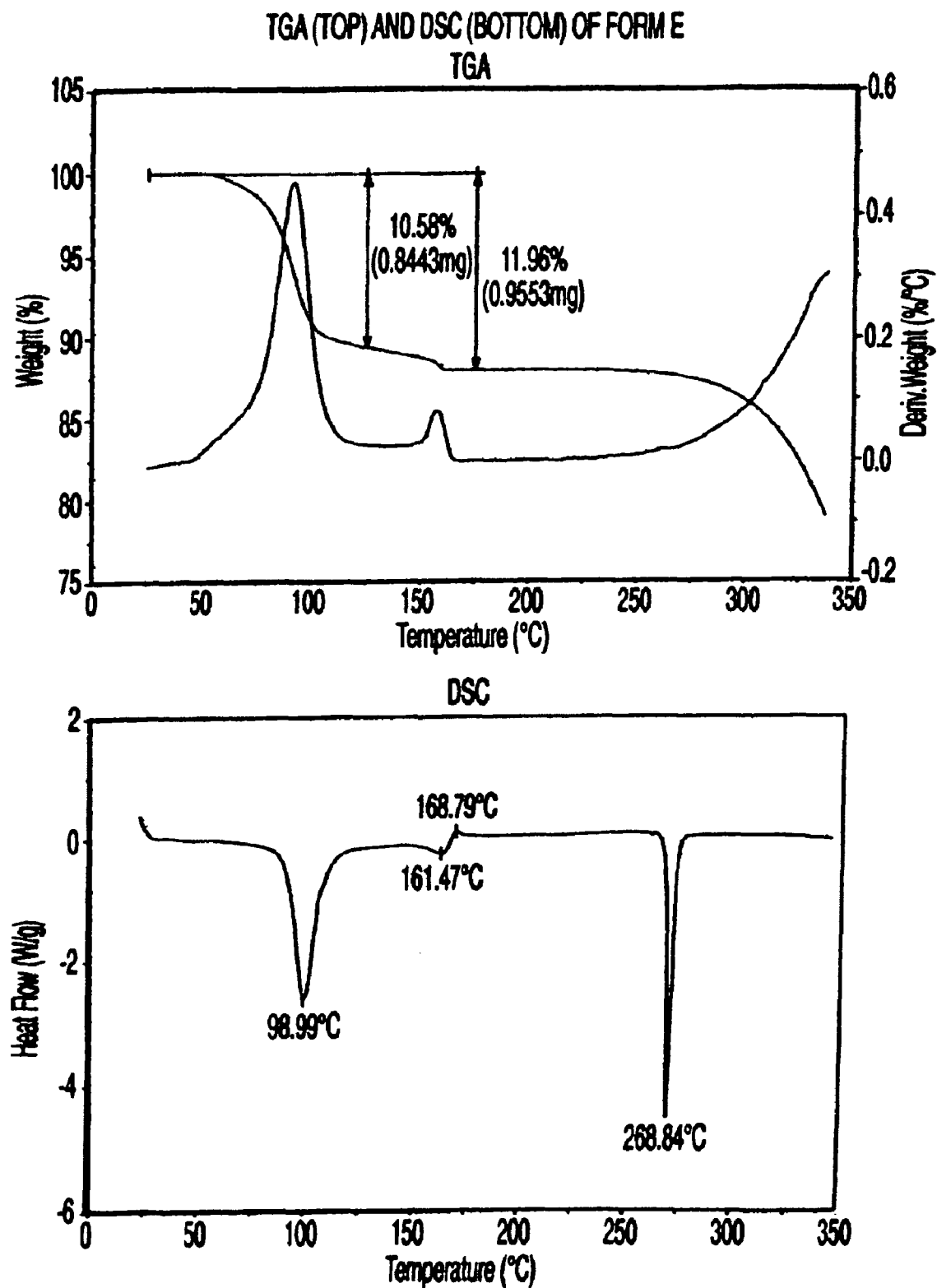
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*Fig. 23*

*Fig. 24*

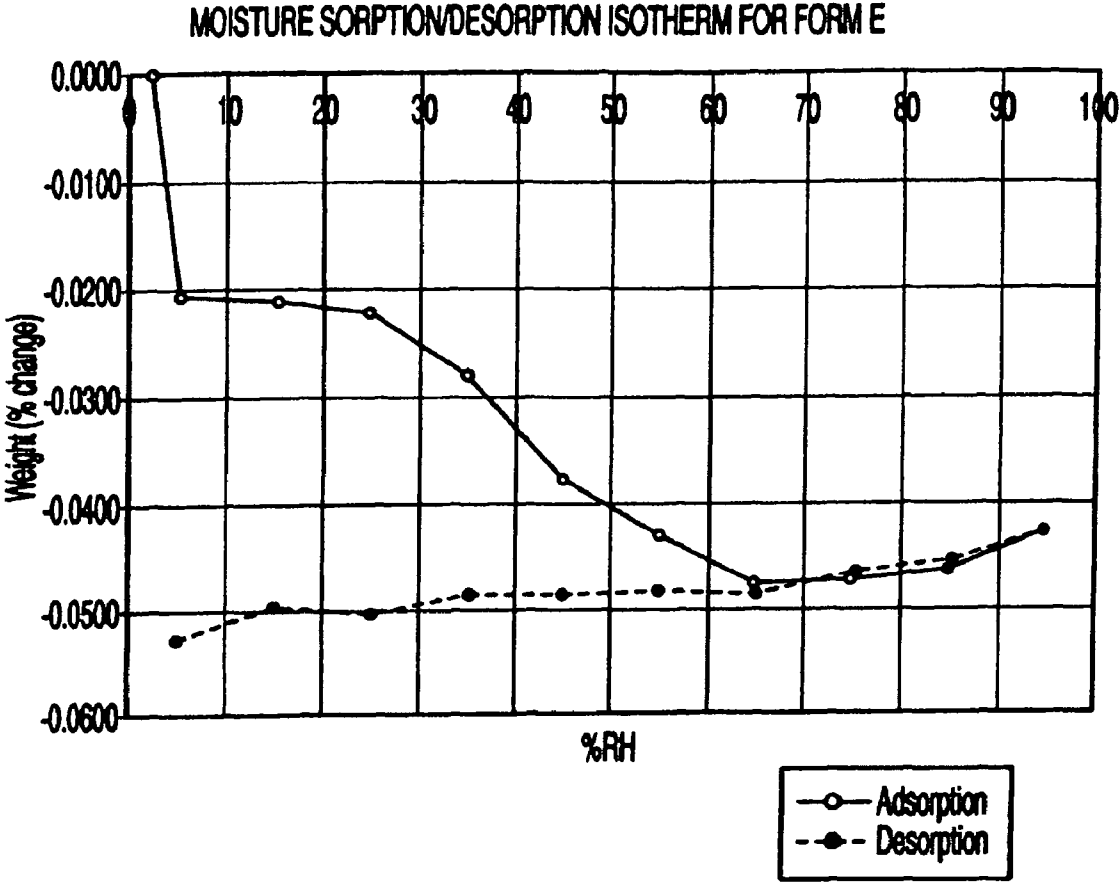


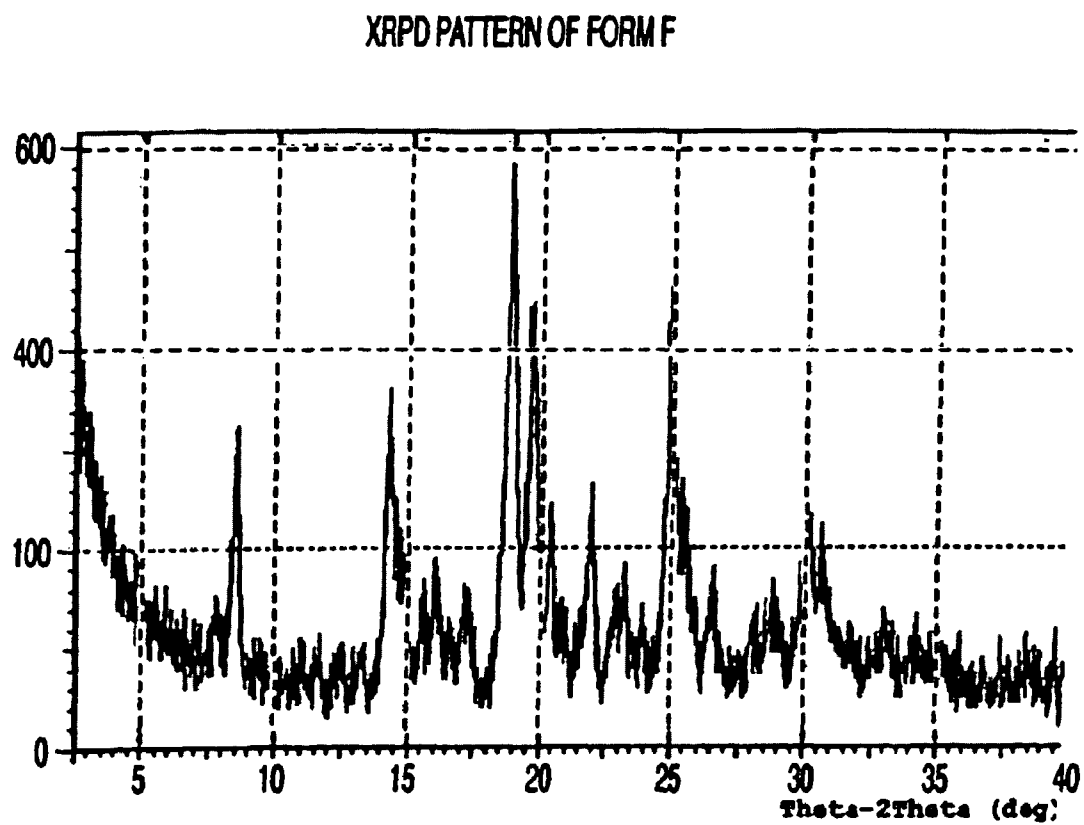
Fig. 25

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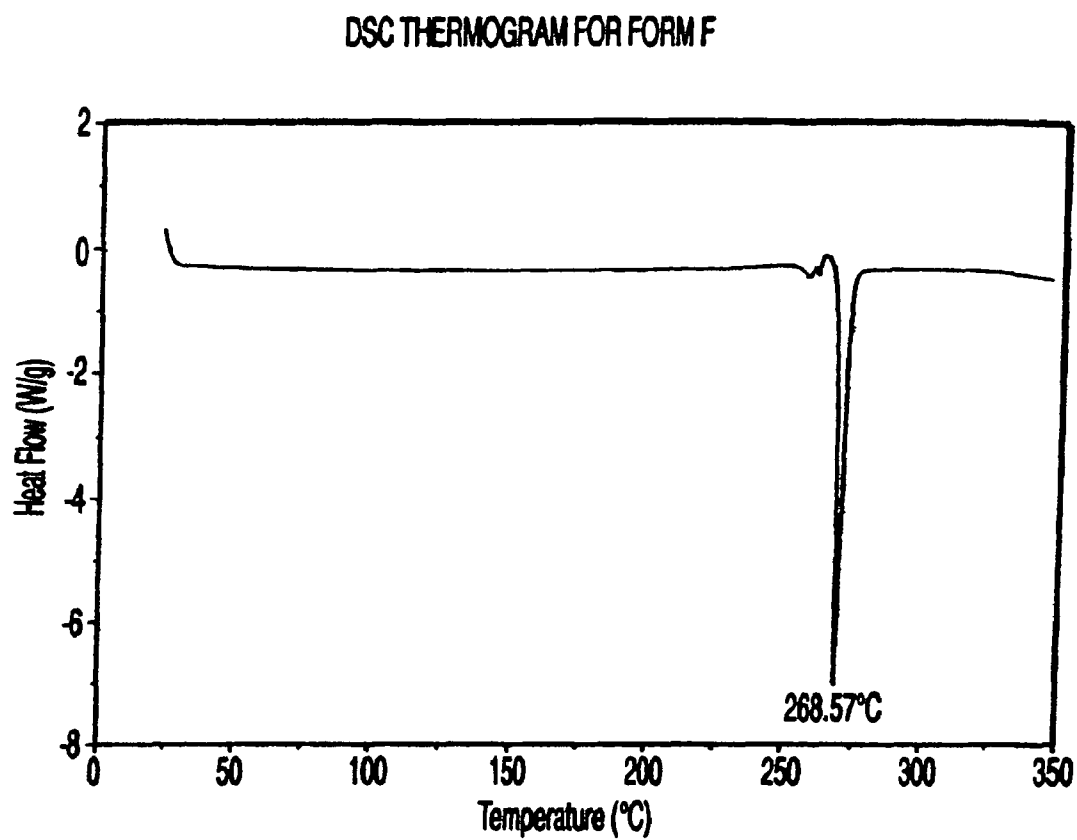
*Fig. 26*

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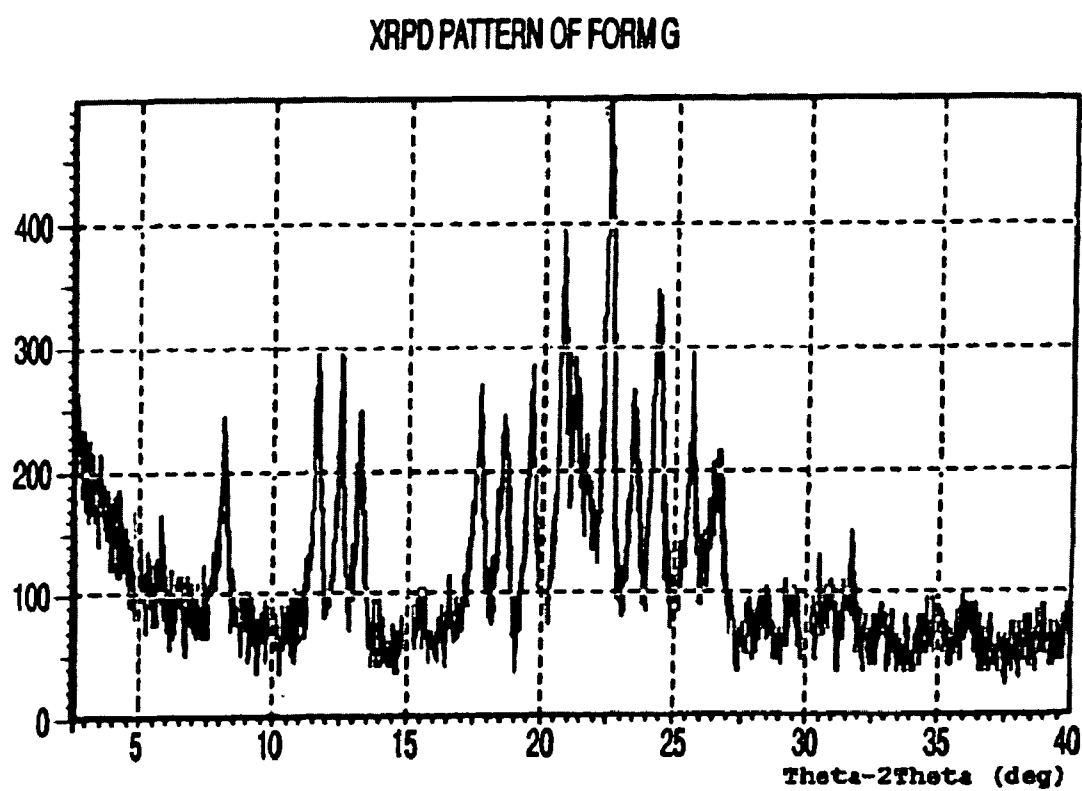
*Fig. 27*

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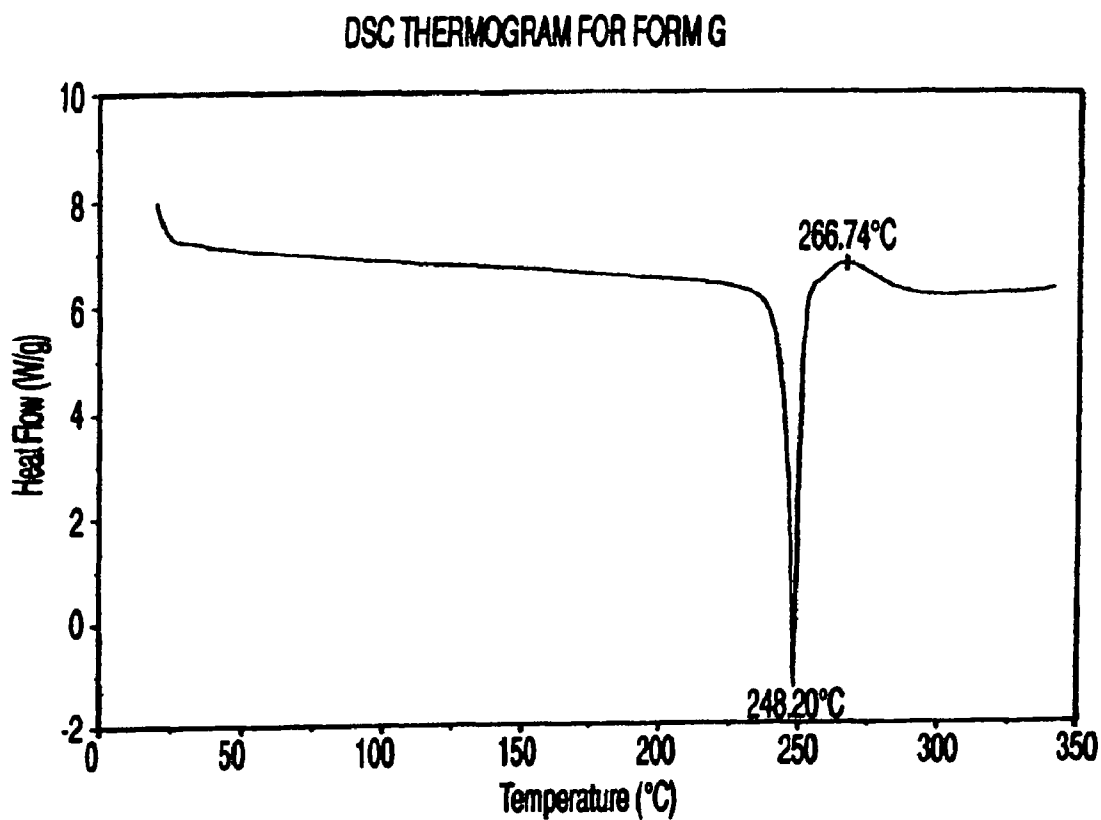
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*Fig. 28*



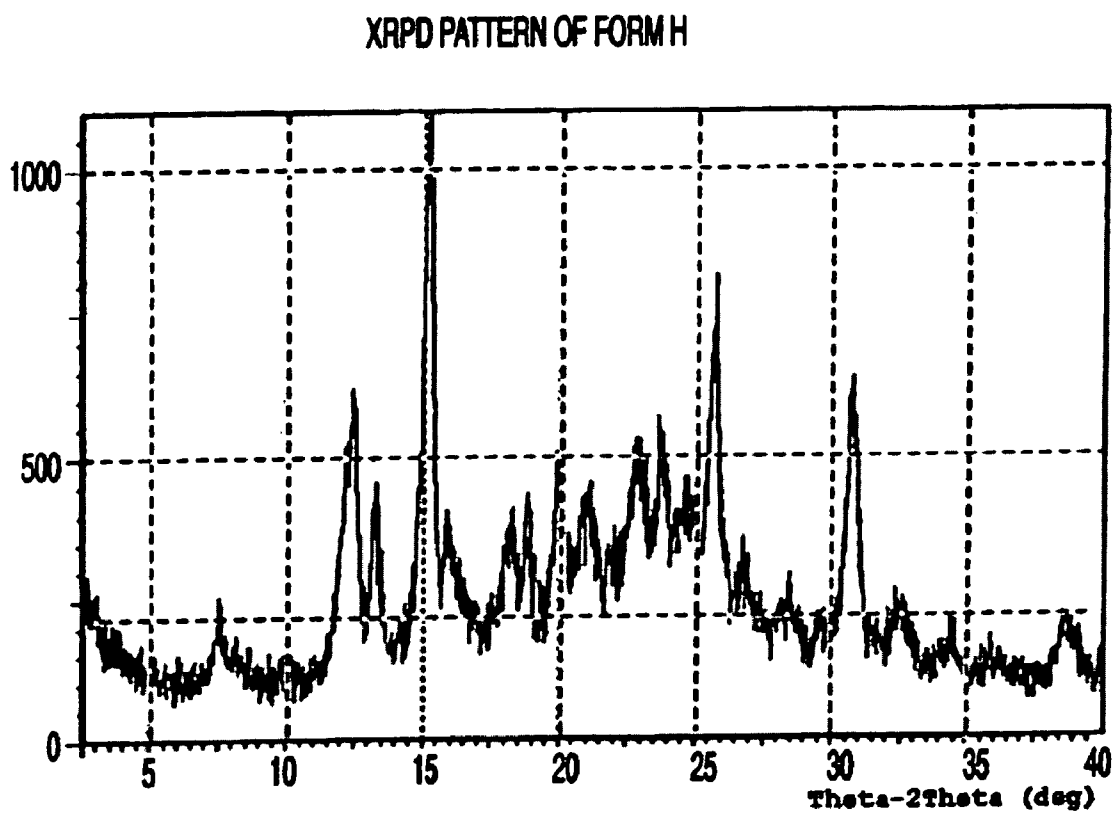
*Fig. 29*

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*Fig. 30*



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## TGA (TOP) AND DSC (BOTTOM) OF FORM H

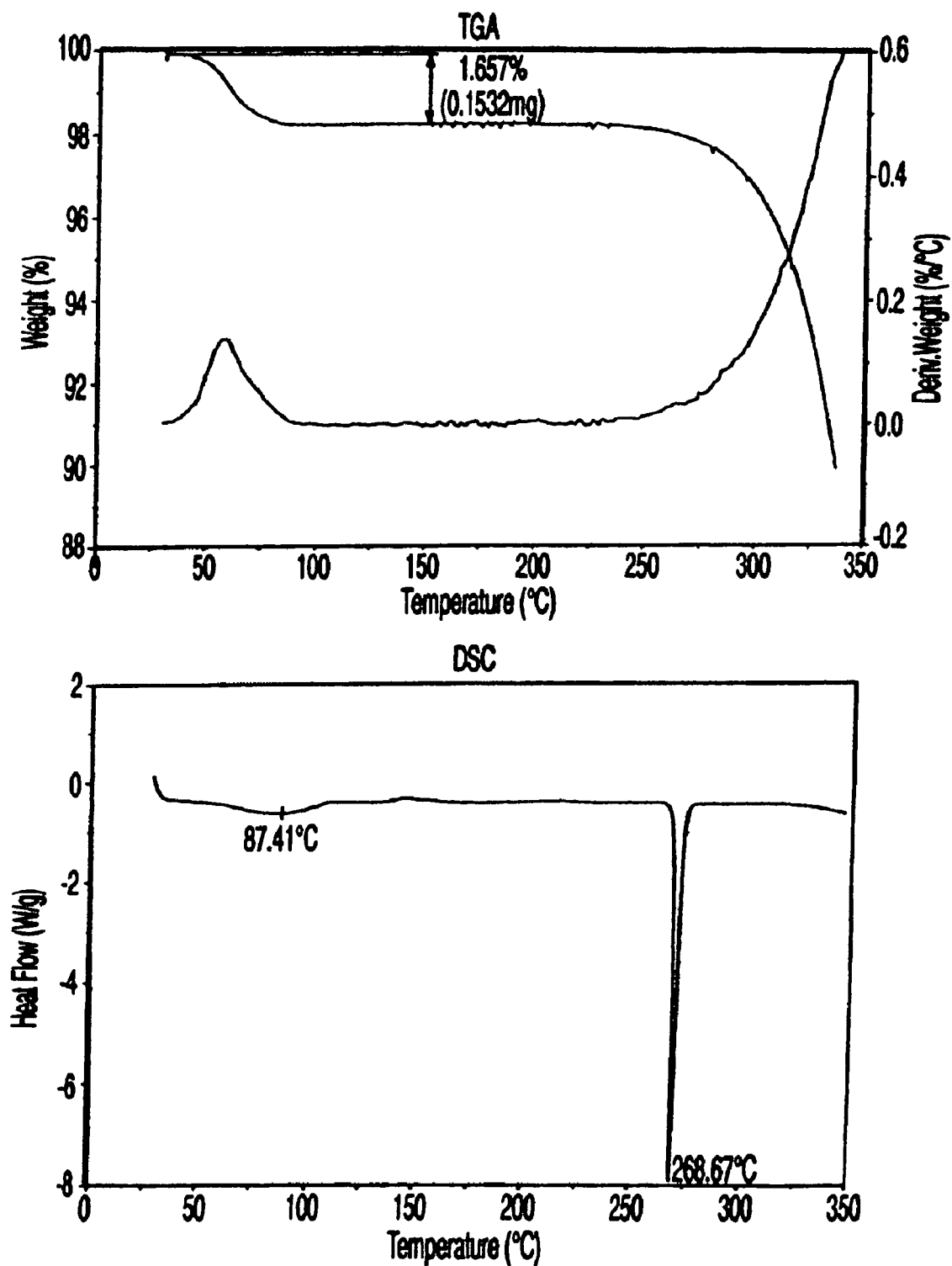
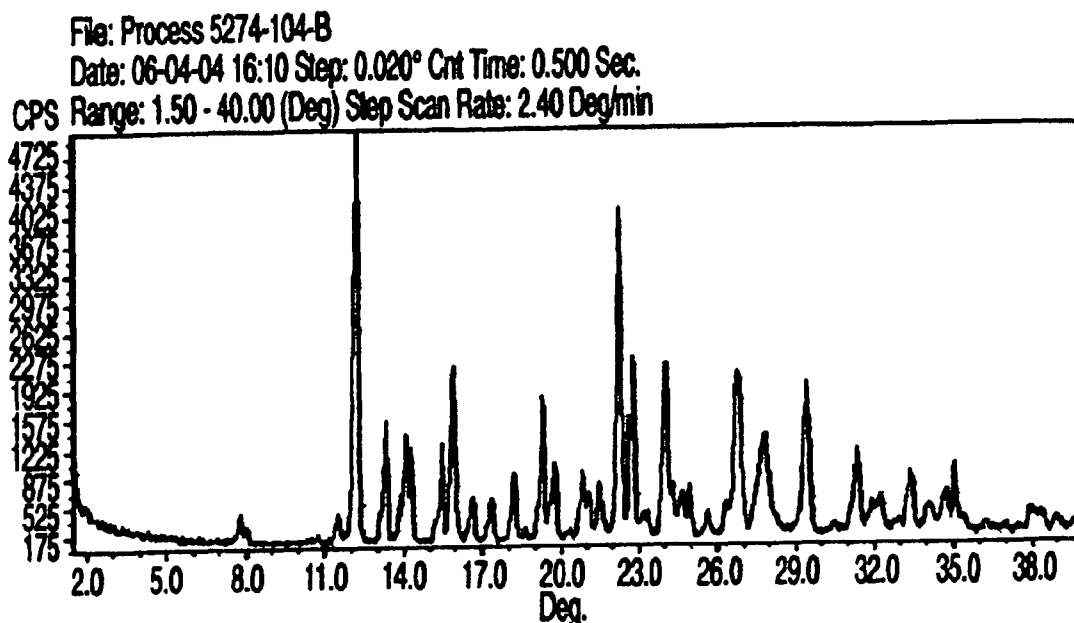
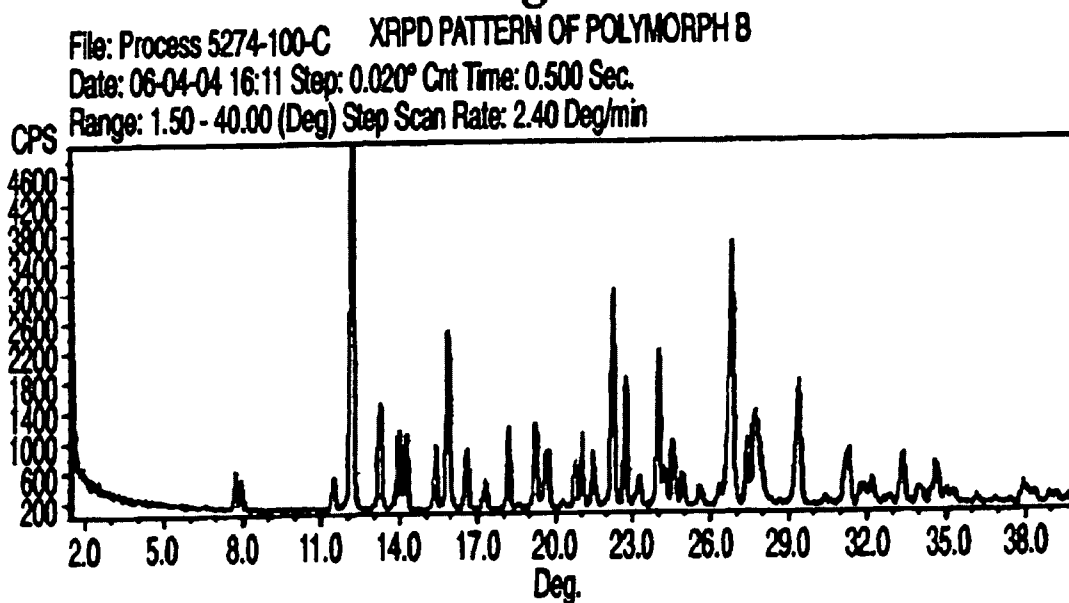


Fig. 31

## XRPD PATTERN OF POLYMORPH B

*Fig. 32**Fig. 33*

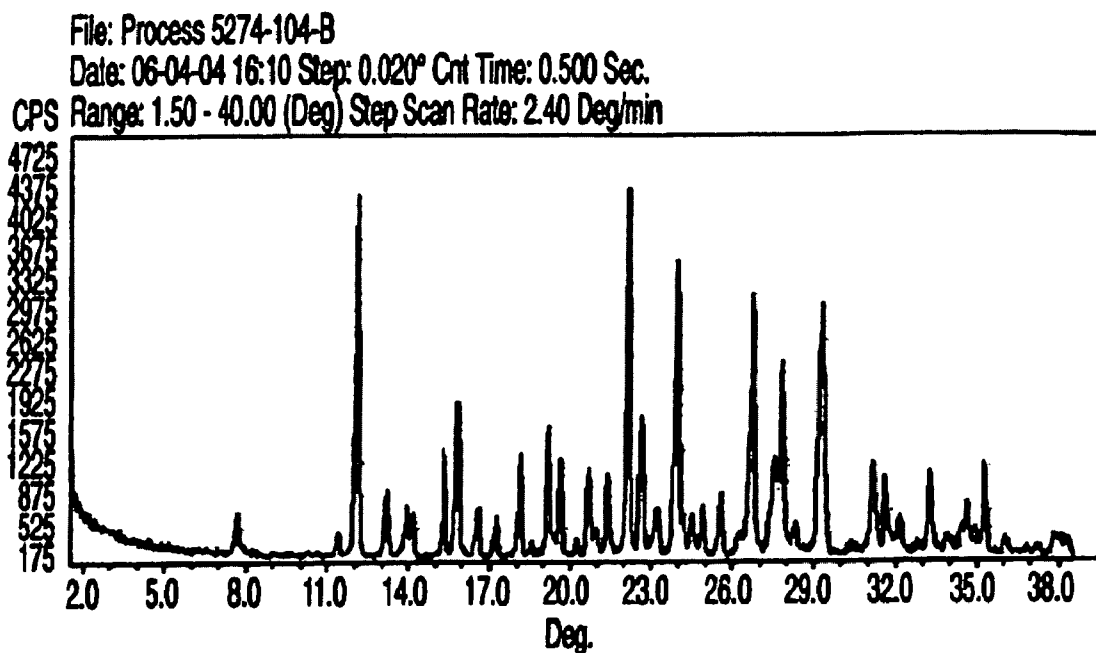
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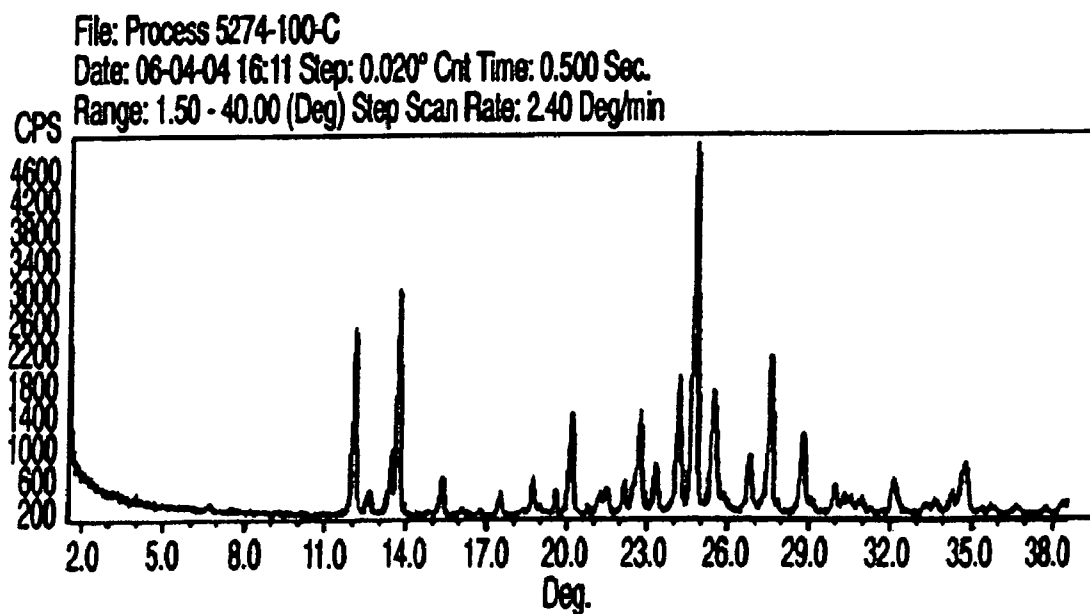
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## XRPD PATTERN OF POLYMORPH B

*Fig. 34*

## XRPD PATTERN OF POLYMORPH E

*Fig. 35*

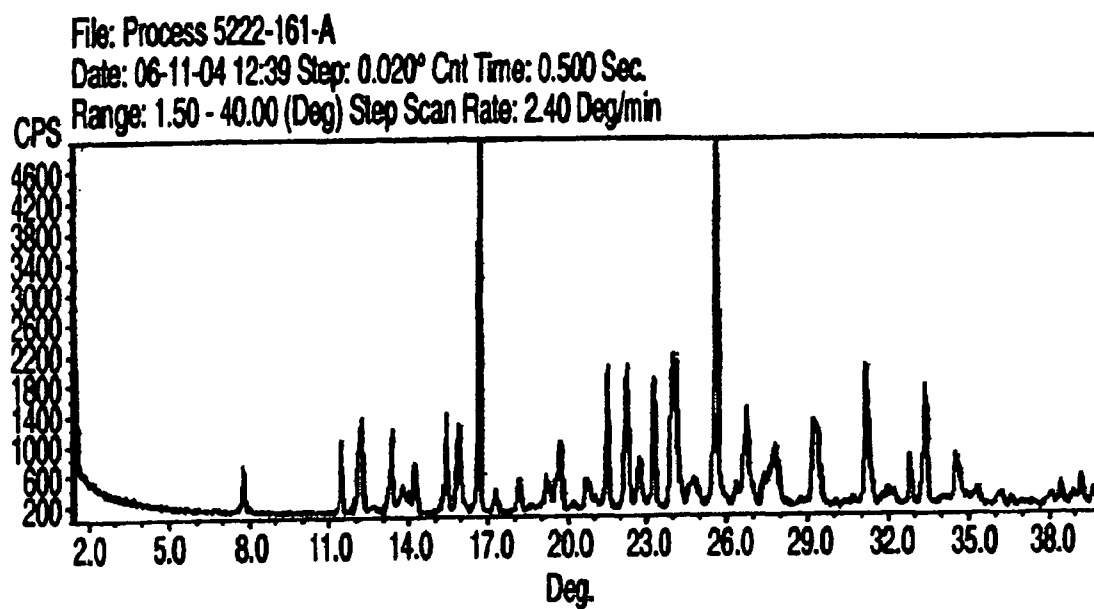
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XRPD PATTERN OF POLYMORPH MIXTURE



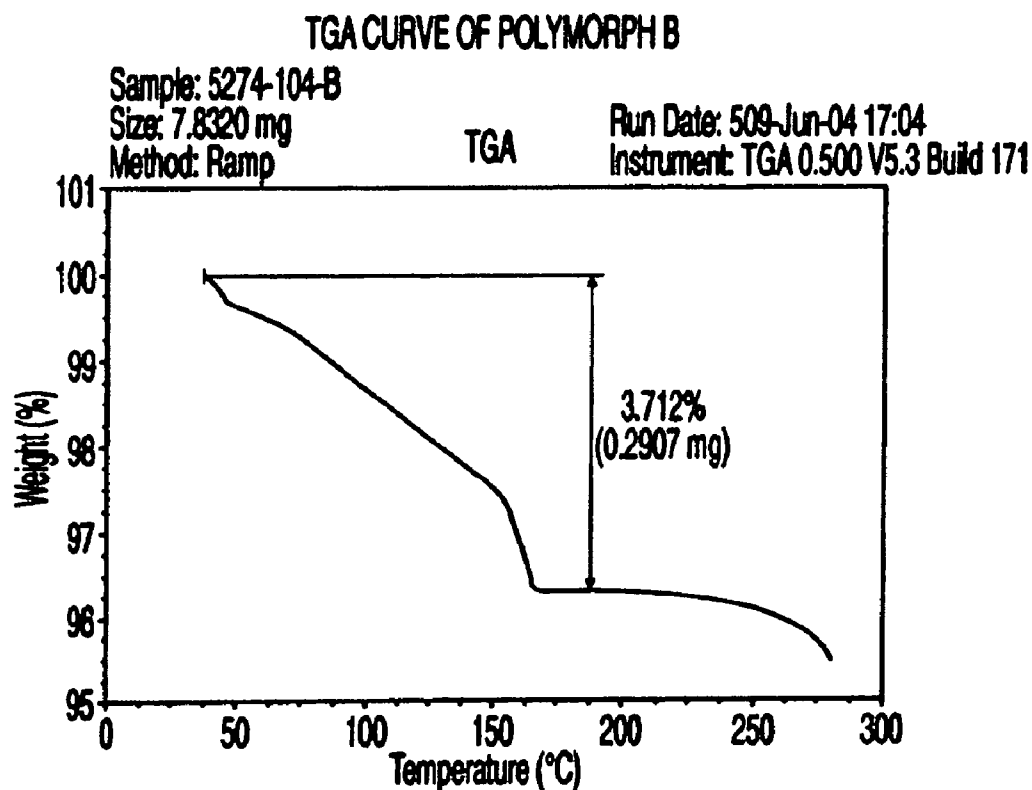
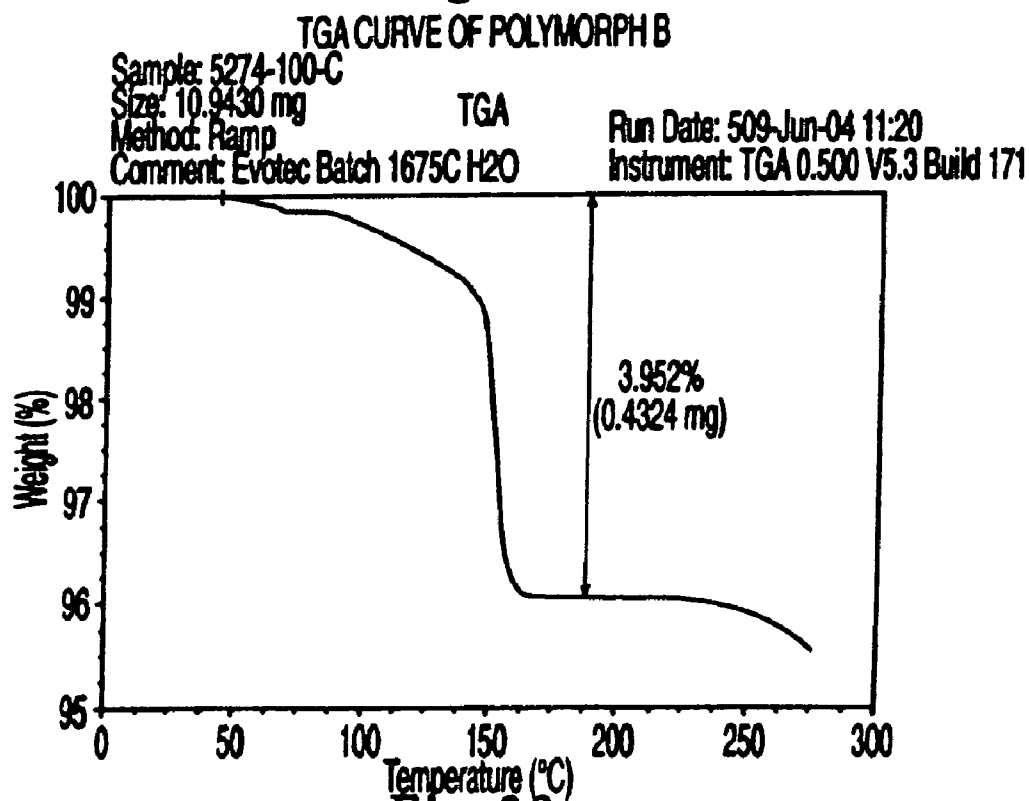
*Fig. 36*

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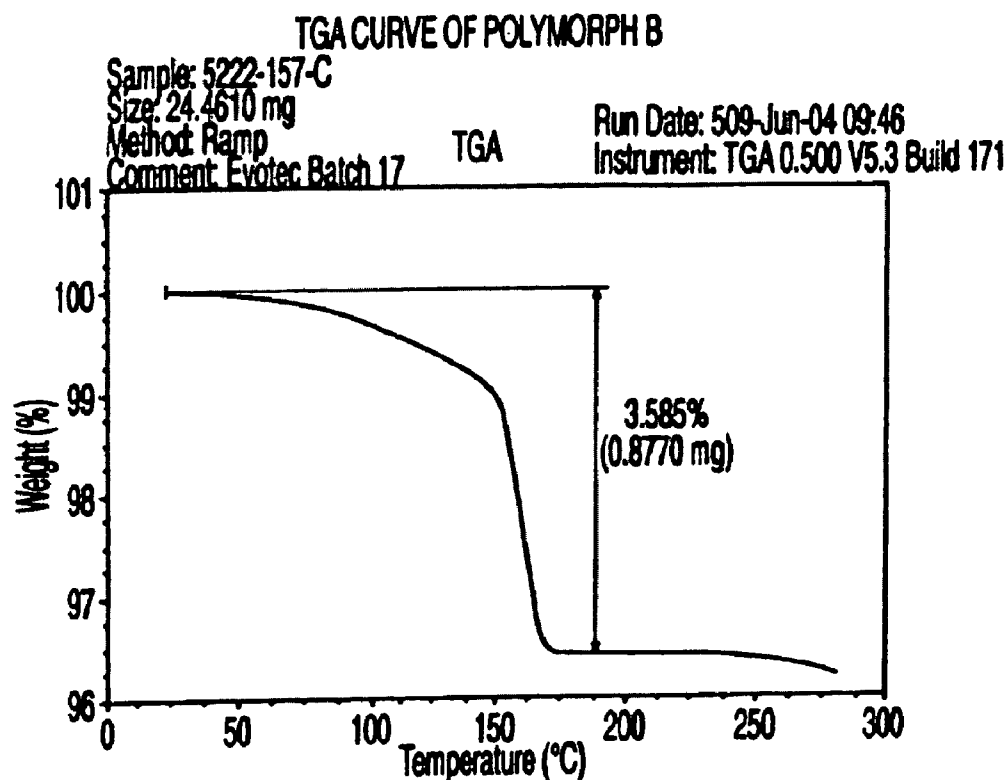
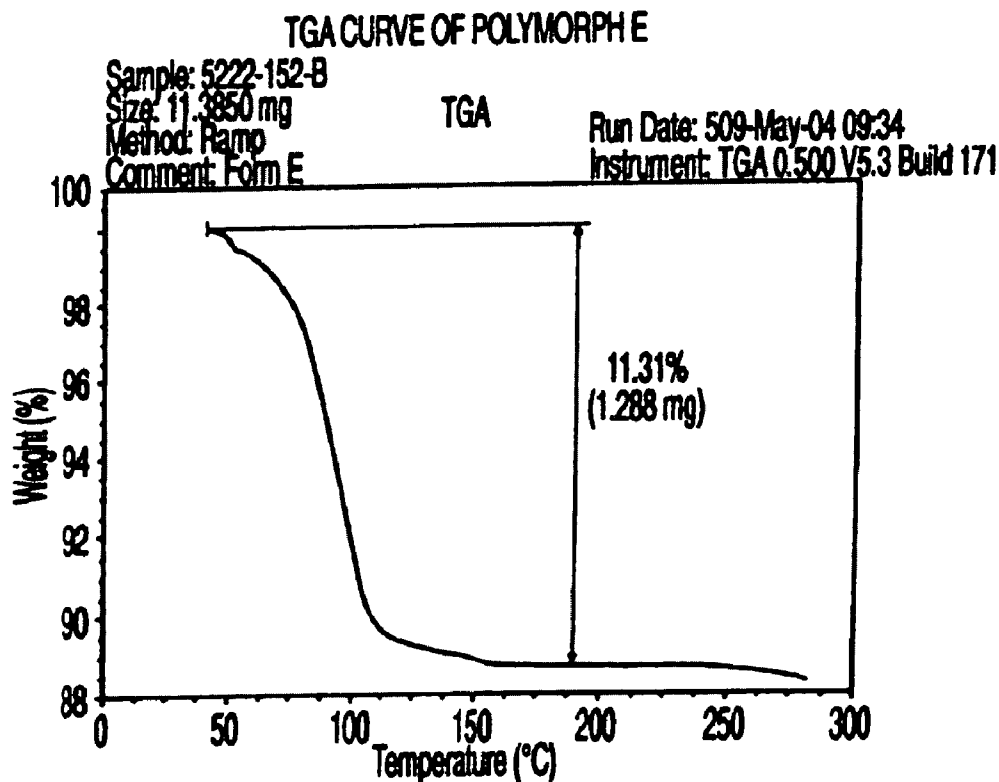
*Fig. 37**Fig. 38*

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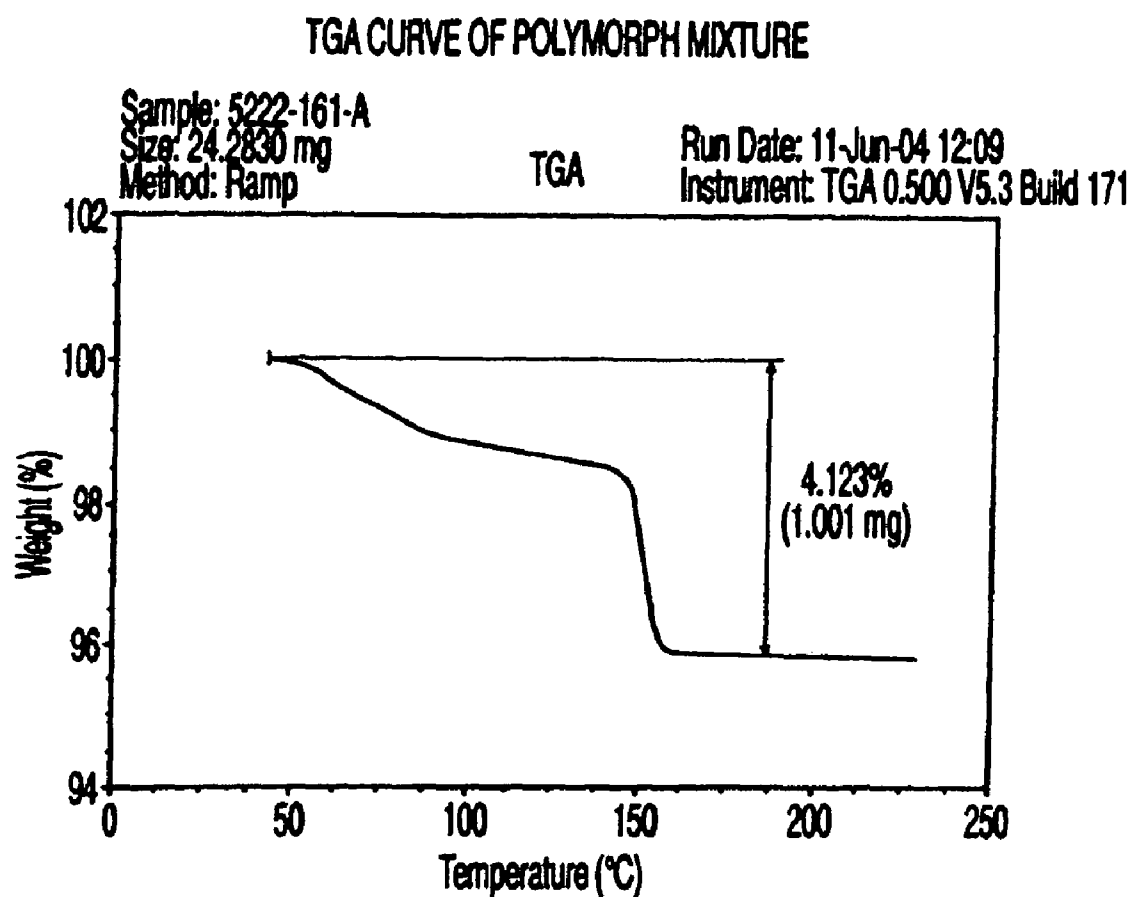
*Fig. 39**Fig. 40*

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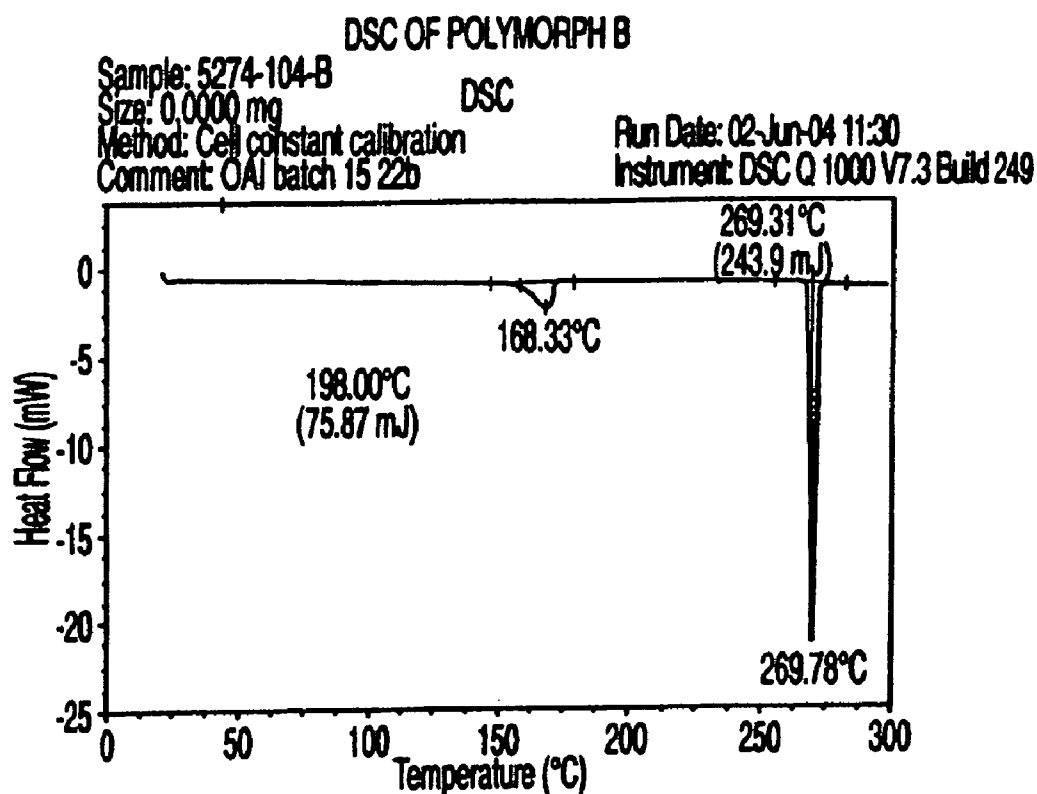
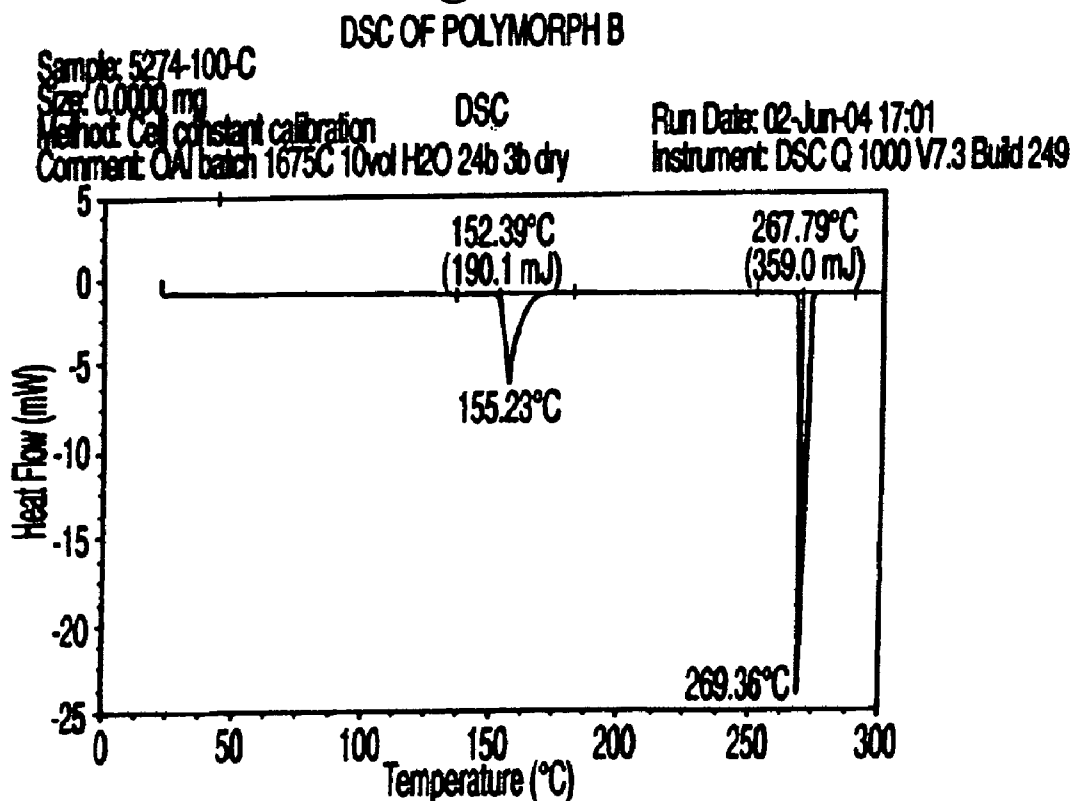
*Fig. 41*

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*Fig. 42**Fig. 43*

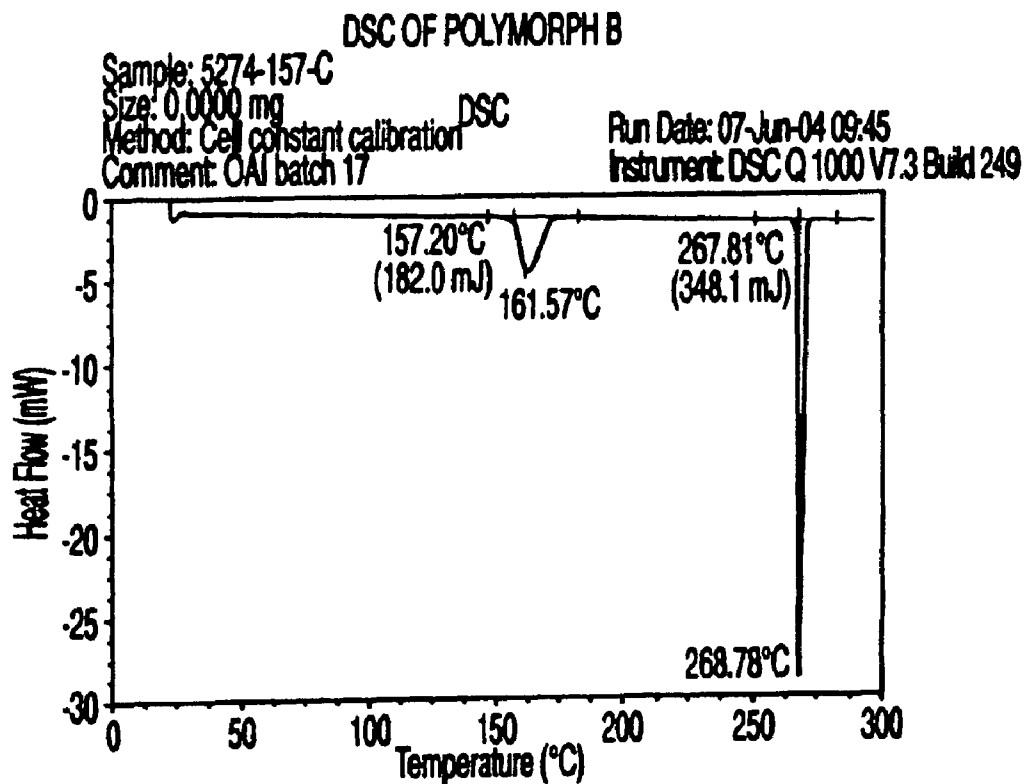
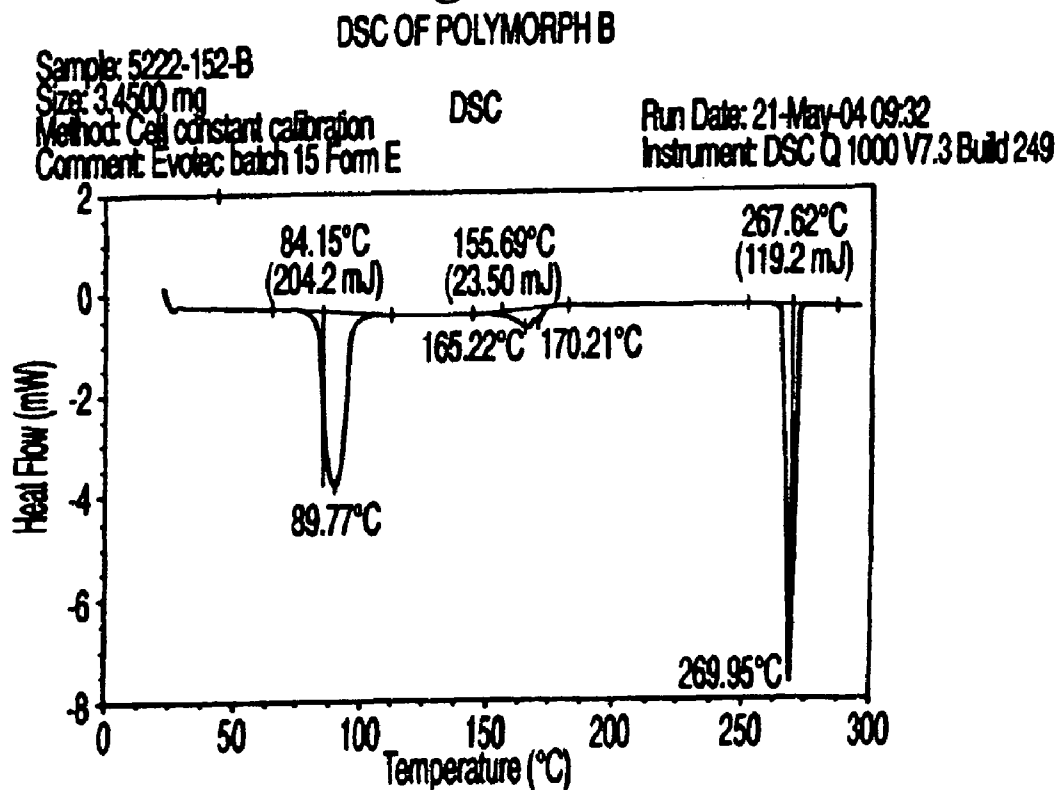


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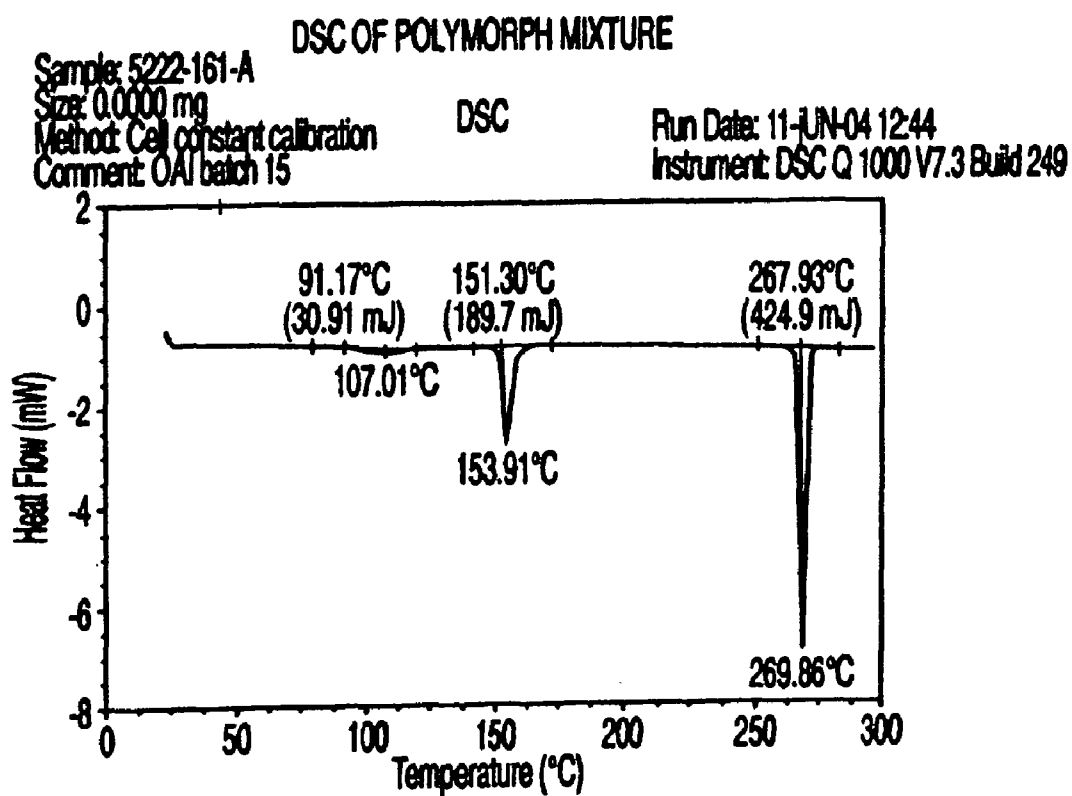
*Fig. 44**Fig. 45*

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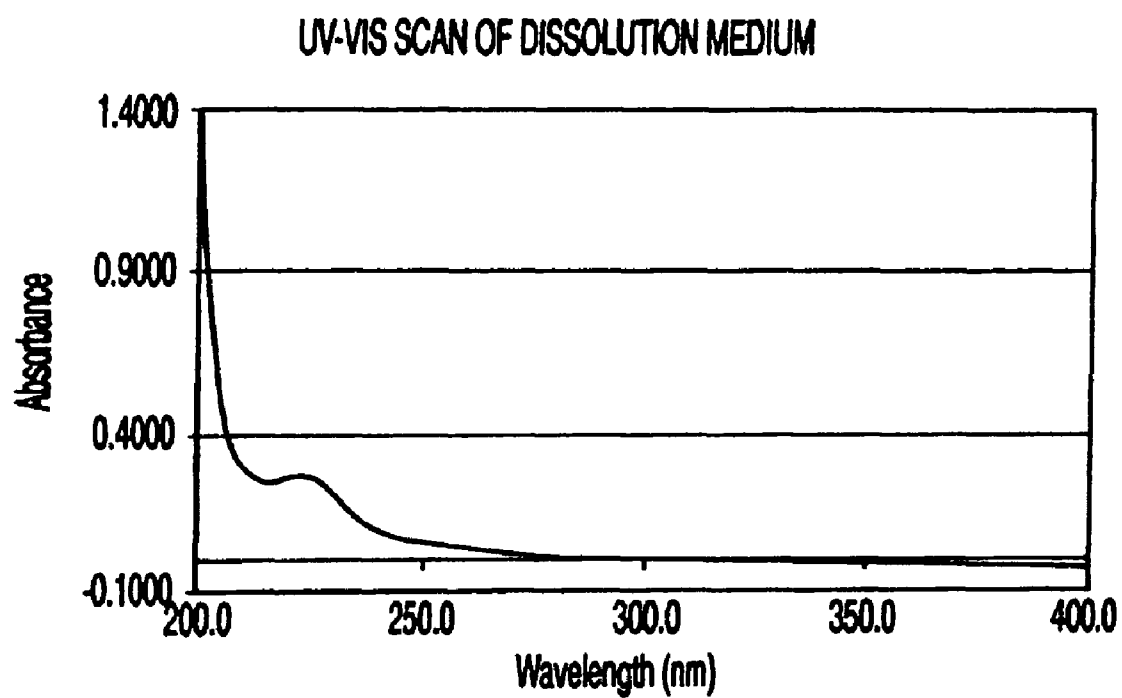
*Fig. 46*

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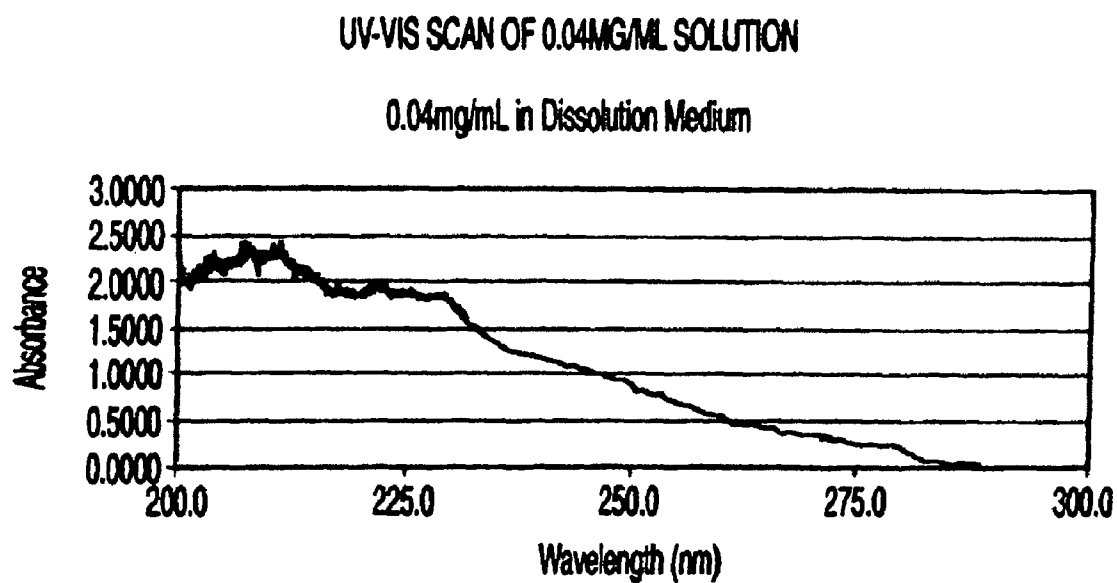
*Fig. 47*

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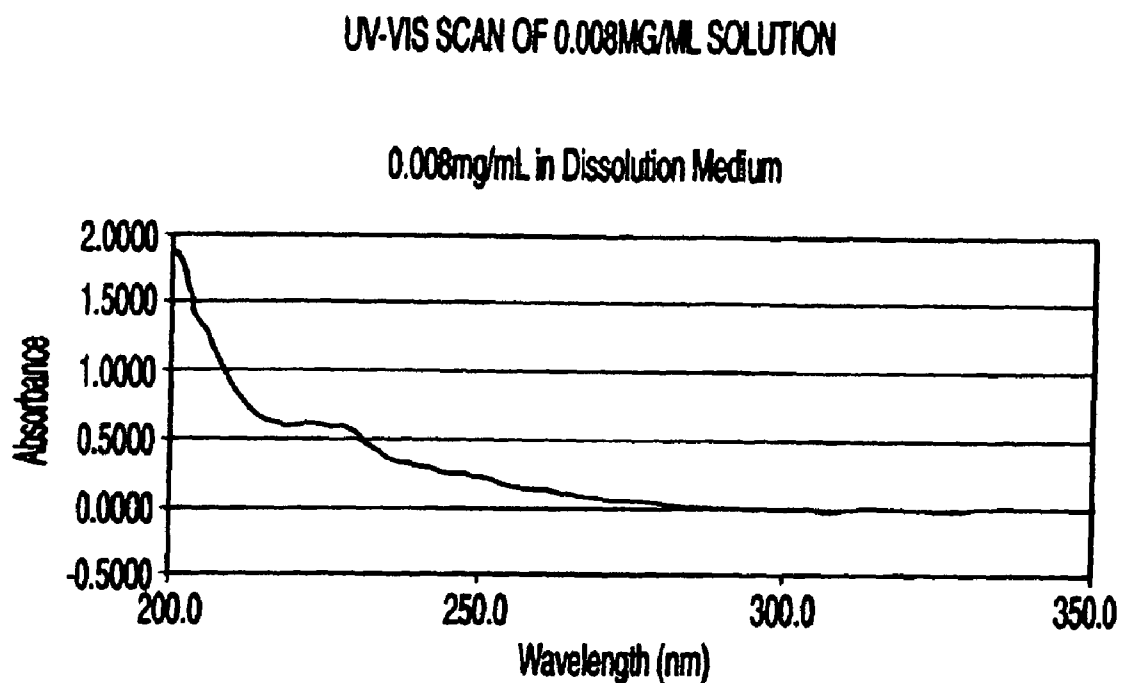
*Fig. 48*

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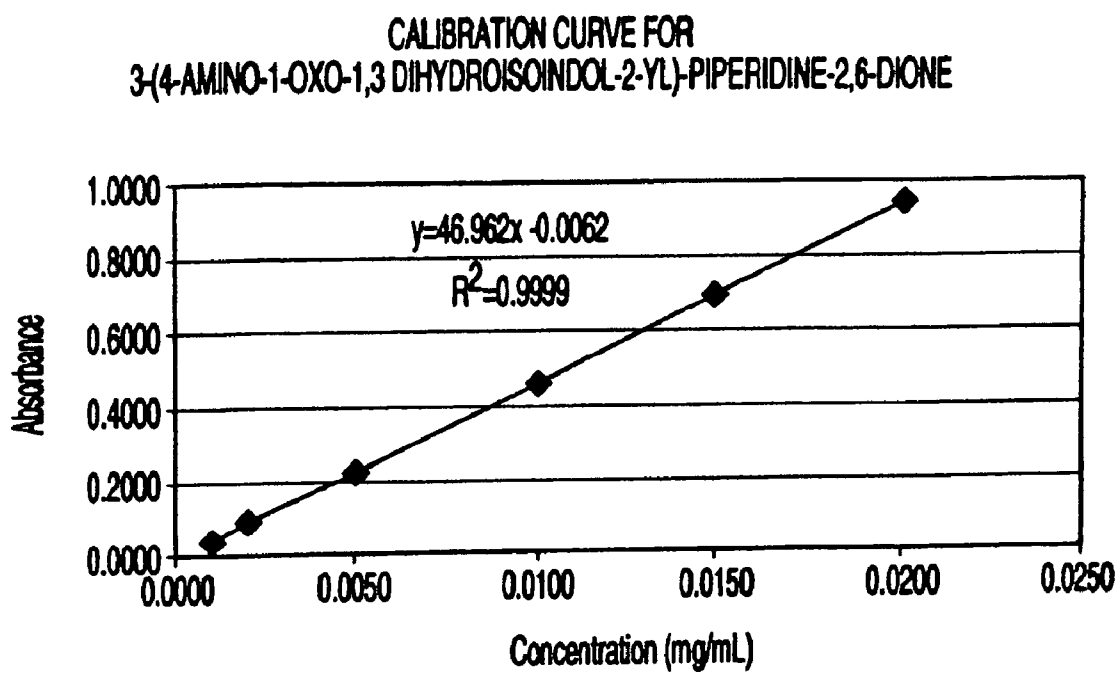
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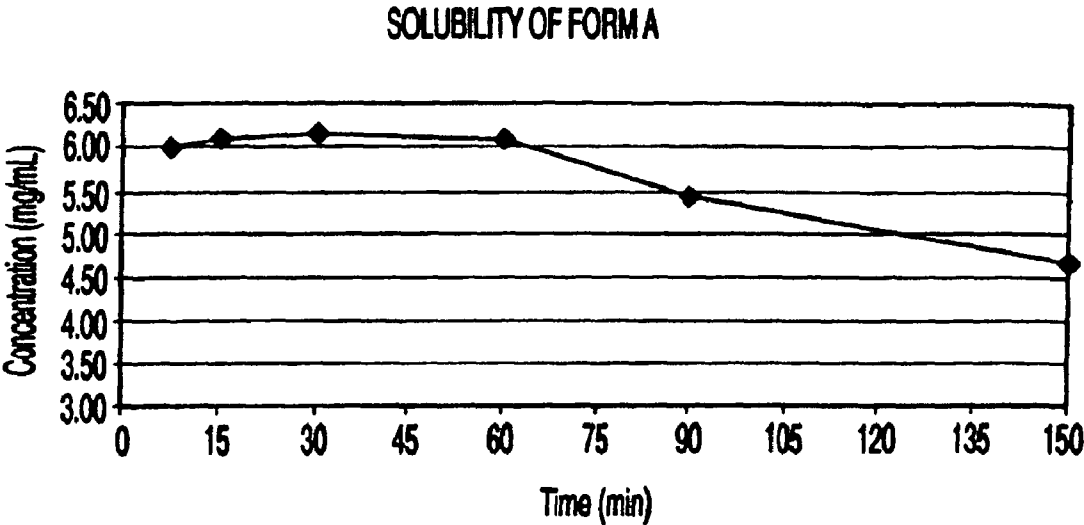
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*Fig. 49*

*Fig. 50*



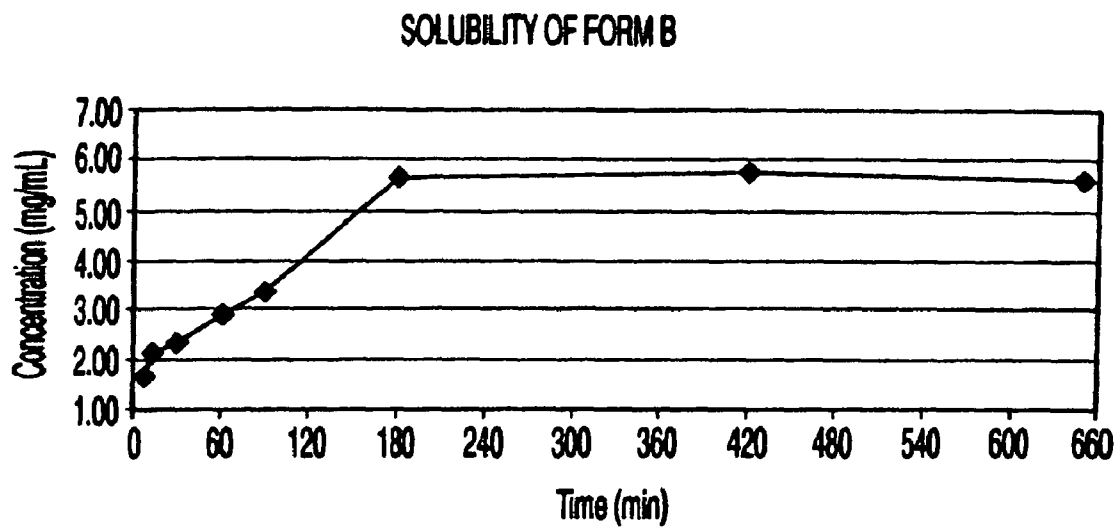
*Fig. 51*

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*Fig. 52*



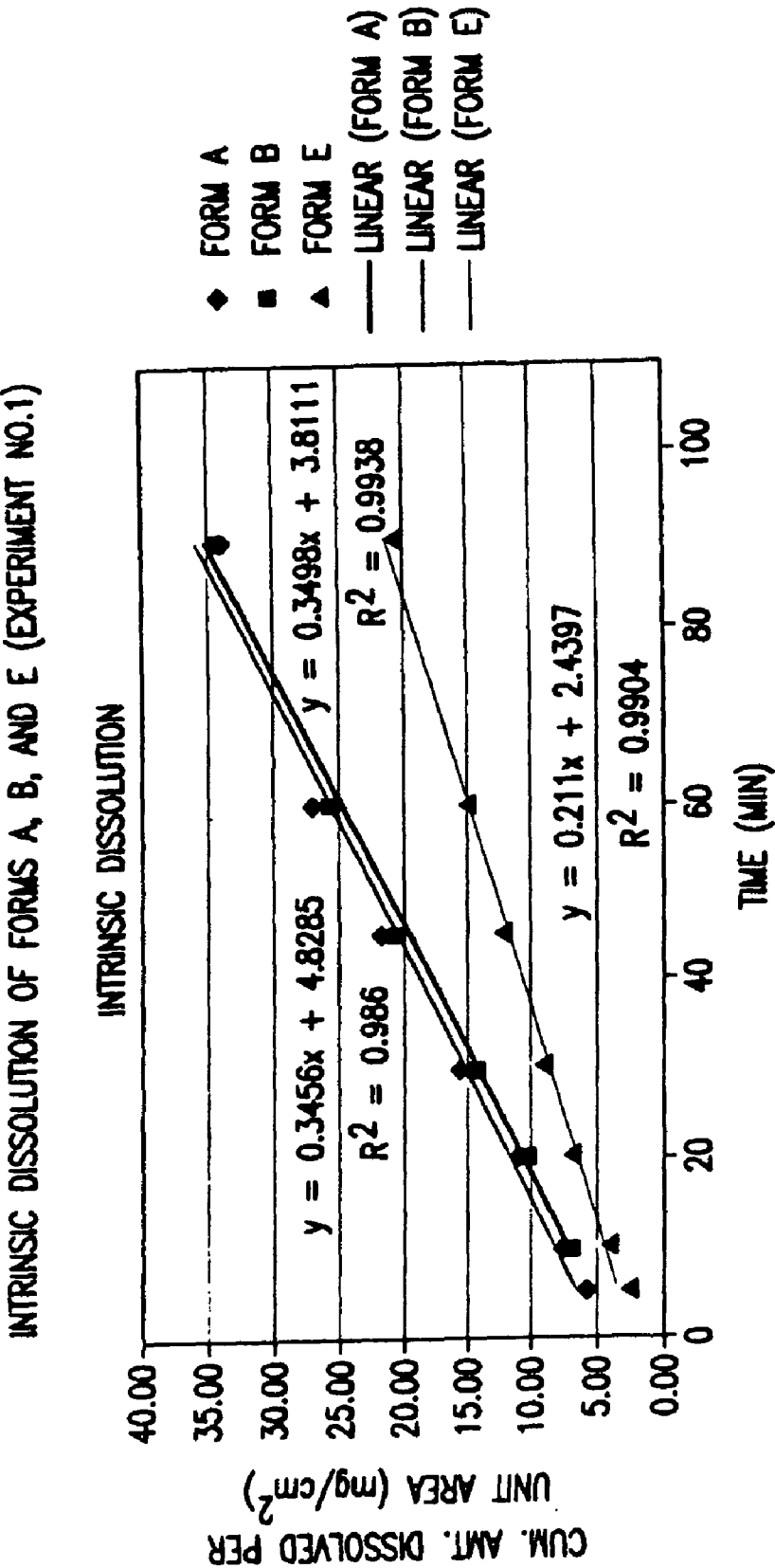


FIG. 53

INTRINSIC DISSOLUTION OF FORMS A, B, AND E (EXPERIMENT NO.2)

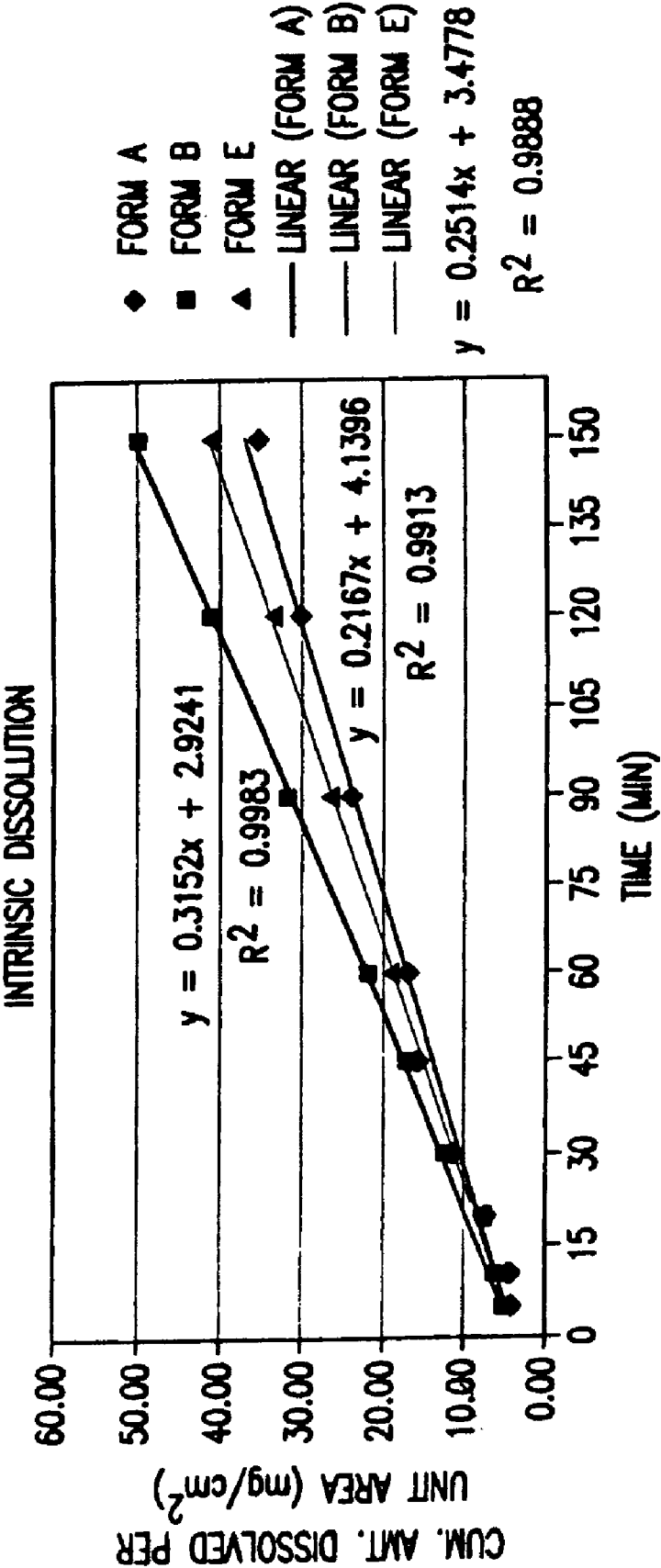


FIG.54

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1

**POLYMORPHIC FORMS OF  
3-(4-AMINO-1-OXO-1,3  
DIHYDRO-ISOINDOL-2-YL)-  
PIPERIDINE-2,6-DIONE**

This application is a divisional application of U.S. patent application Ser. No. 10/934,863, filed Sep. 3, 2004, now U.S. Pat. No. 7,465,800 presently pending, which claims the benefit of U.S. provisional application 60/499,723, filed Sep. 4, 2003, the contents of each of which are incorporated by reference herein in their entireties.

**1. FIELD OF THE INVENTION**

This invention relates to polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, compositions comprising the polymorphic forms, methods of making the polymorphic forms and methods of their use for the treatment of diseases and conditions including, but not limited to, inflammatory diseases, autoimmune diseases, and cancer.

**2. BACKGROUND OF THE INVENTION**

Many compounds can exist in different crystal forms, or polymorphs, which exhibit different physical, chemical, and spectroscopic properties. For example, certain polymorphs of a compound may be more readily soluble in particular solvents, may flow more readily, or may compress more easily than others. See, e.g., P. DiMartino, et al., *J. Thermal Anal.*, 48:447-458 (1997). In the case of drugs, certain solid forms may be more bioavailable than others, while others may be more stable under certain manufacturing, storage, and biological conditions. This is particularly important from a regulatory standpoint, since drugs are approved by agencies such as the U.S. Food and Drug Administration only if they meet exacting purity and characterization standards. Indeed, the regulatory approval of one polymorph of a compound, which exhibits certain solubility and physico-chemical (including spectroscopic) properties, typically does not imply the ready approval of other polymorphs of that same compound.

Polymorphic forms of a compound are known in the pharmaceutical arts to affect, for example, the solubility, stability, flowability, fractability, and compressibility of the compound, as well as the safety and efficacy of drug products comprising it. See, e.g., Knapman, K. *Modern Drug Discoveries*, 2000, 53. Therefore, the discovery of new polymorphs of a drug can provide a variety of advantages.

U.S. Pat. Nos. 5,635,517 and 6,281,230, both to Muller et al., disclose 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, which is useful in treating and preventing a wide range of diseases and conditions including, but not limited to, inflammatory diseases, autoimmune diseases, and cancer. New polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can further the development of formulations for the treatment of these chronic illnesses, and may yield numerous formulation, manufacturing and therapeutic benefits.

**3. SUMMARY OF THE INVENTION**

This invention encompasses polymorphs of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. In certain aspects, the invention provides polymorphs of the compound identified herein as forms A, B, C, D, E, F, G, and H. The invention also encompasses mixtures of these forms. In further embodiments, this invention provides methods of making, isolating and characterizing the polymorphs.

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This invention also provides pharmaceutical compositions and single unit dosage forms comprising a polymorph of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The invention further provides methods for the treatment or prevention of a variety of diseases and disorders, which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of a polymorph of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

**4. BRIEF DESCRIPTION OF THE DRAWINGS**

Specific aspects of the invention can be understood with reference to the attached figures:

FIG. 1 provides a representative X-ray powder diffraction (XRPD) pattern of Form A;

FIG. 2 provides a representative IR spectrum of Form A;

FIG. 3 provides a representative Raman spectrum of Form A;

FIG. 4 provides a representative thermogravimetric analysis (TGA) curve and a representative differential scanning calorimeter (DSC) thermogram of Form A;

FIG. 5 provides a representative moisture sorption/desorption isotherm of Form A;

FIG. 6 provides a representative XRPD pattern of Form B;

FIG. 7 provides a representative IR spectrum of Form B;

FIG. 8 provides a representative Raman spectrum of Form B;

FIG. 9 provides a representative TGA curve and a representative DSC thermogram of Form B;

FIG. 10 provides representative TG-IR results of Form B;

FIG. 11 provides a representative moisture sorption/desorption isotherm of Form B;

FIG. 12 provides a representative XRPD pattern of Form C;

FIG. 13 provides a representative IR spectrum of Form C;

FIG. 14 provides a representative Raman spectrum of Form C;

FIG. 15 provides a representative TGA curve and a representative DSC thermogram of Form C;

FIG. 16 provides representative TG-IR results of Form C;

FIG. 17 provides a representative moisture sorption/desorption isotherm of Form C;

FIG. 18 provides a representative XRPD pattern of Form D;

FIG. 19 provides a representative IR spectrum of Form D;

FIG. 20 provides a representative Raman spectrum of Form D;

FIG. 21 provides a representative TGA curve and a representative DSC thermogram of Form D;

FIG. 22 provides a representative moisture sorption/desorption isotherm of Form D;

FIG. 23 provides a representative XRPD pattern of Form E;

FIG. 24 provides a representative TGA curve and a representative DSC thermogram of Form E;

FIG. 25 provides a representative moisture sorption/desorption isotherm of Form E;

FIG. 26 provides a representative XRPD pattern for a sample of Form F;

FIG. 27 provides a representative thermogram of Form F;

FIG. 28 provides a representative XRPD pattern of Form G;

FIG. 29 provides a representative DSC thermogram for a sample of Form G;

FIG. 30 provides a representative XRPD pattern of Form H;

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FIG. 31 provides a representative TGA curve and a representative DSC thermogram of Form H;

FIG. 32 provides a representative XRPD pattern of Form B;

FIG. 33 provides a representative XRPD pattern of Form B;

FIG. 34 provides a representative XRPD pattern of Form B;

FIG. 35 provides a representative XRPD pattern of Form E;

FIG. 36 provides a representative XRPD pattern of polymorph mixture;

FIG. 37 provides a representative TGA curve of Form B;

FIG. 38 provides a representative TGA curve of Form B;

FIG. 39 provides a representative TGA curve of Form B;

FIG. 40 provides a representative TGA curve of Form E;

FIG. 41 provides a representative TGA curve of polymorph mixture;

FIG. 42 provides a representative DSC thermogram of Form B;

FIG. 43 provides a representative DSC thermogram of Form B;

FIG. 44 provides a representative DSC thermogram of Form B;

FIG. 45 provides a representative DSC thermogram of Form E;

FIG. 46 provides a representative DSC thermogram of polymorph mixture;

FIG. 47 provides a UV-Vis scan of dissolution medium;

FIG. 48 provides a UV-Vis scan of 0.04 mg/ml of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in dissolution medium;

FIG. 49 provides a UV-Vis scan of 0.008 mg/ml of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in dissolution medium;

FIG. 50 provides a calibration curve for 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione;

FIG. 51 provides a solubility curve of Form A;

FIG. 52 provides a solubility curve of Form B;

FIG. 53 provides an intrinsic dissolution of Forms A, B and E; and

FIG. 54 provides an intrinsic dissolution of Forms A, B and E.

## 5. DETAILED DESCRIPTION OF THE INVENTION

### 5.1 Definitions

As used herein and unless otherwise indicated, the terms “treat,” “treating” and “treatment” refer to the alleviation of a disease or disorder and/or at least one of its attendant symptoms.

As used herein and unless otherwise indicated, the terms “prevent,” “preventing” and “prevention” refer to the inhibition of a symptom of a disease or disorder or the disease itself.

As used herein and unless otherwise indicated, the terms “polymorph” and “polymorphic form” refer to solid crystalline forms of a compound or complex. Different polymorphs of the same compound can exhibit different physical, chemical and/or spectroscopic properties. Different physical properties include, but are not limited to stability (e.g., to heat or light), compressibility and density (important in formulation and product manufacturing), and dissolution rates (which can affect bioavailability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical characteristics (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both

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(e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). Different physical properties of polymorphs can affect their processing. For example, one polymorph might be more likely to form solvates or might be more difficult to filter or wash free of impurities than another due to, for example, the shape or size distribution of particles of it.

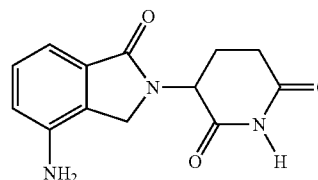
Polymorphs of a molecule can be obtained by a number of methods known in the art. Such methods include, but are not limited to, melt recrystallization, melt cooling, solvent recrystallization, desolvation, rapid evaporation, rapid cooling, slow cooling, vapor diffusion and sublimation. Polymorphs can be detected, identified, classified and characterized using well-known techniques such as, but not limited to, differential scanning calorimetry (DSC), thermogravimetry (TGA), X-ray powder diffractometry (XRPD), single crystal X-ray diffractometry, vibrational spectroscopy, solution calorimetry, solid state nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, Raman spectroscopy, hot stage optical microscopy, scanning electron microscopy (SEM), electron crystallography and quantitative analysis, particle size analysis (PSA), surface area analysis, solubility, and rate of dissolution.

As used herein to refer to the spectra or data presented in graphical form (e.g., XRPD, IR, Raman and NMR spectra), and unless otherwise indicated, the term “peak” refers to a peak or other special feature that one skilled in the art would recognize as not attributable to background noise. The term “significant peaks” refers to peaks at least the median size (e.g., height) of other peaks in the spectrum or data, or at least 1.5, 2, or 2.5 times the median size of other peaks in the spectrum or data.

As used herein and unless otherwise indicated, the term “substantially pure” when used to describe a polymorph of a compound means a solid form of the compound that comprises that polymorph and is substantially free of other polymorphs of the compound. A representative substantially pure polymorph comprises greater than about 80% by weight of one polymorphic form of the compound and less than about 20% by weight of other polymorphic forms of the compound, more preferably greater than about 90% by weight of one polymorphic form of the compound and less than about 10% by weight of the other polymorphic forms of the compound, even more preferably greater than about 95% by weight of one polymorphic form of the compound and less than about 5% by weight of the other polymorphic forms of the compound, and most preferably greater than about 97% by weight of one polymorphic forms of the compound and less than about 3% by weight of the other polymorphic forms of the compound.

### 5.2 Polymorphic Forms

This invention is directed to polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione, which has the structure shown below:



This compound can be prepared according to the methods described in U.S. Pat. Nos. 6,281,230 and 5,635,517, the entireties of which are incorporated herein by reference. For

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example, the compound can be prepared through catalytic hydrogenation of 3-(4-nitro-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. 3-(4-Nitro-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can be obtained by allowing 2,6-dioxopiperidin-3-ammonium chloride to react with methyl 2-bromomethyl-4-nitrobenzoate in dimethylformamide in the presence of triethylamine. The methyl 2-bromomethyl-4-nitrobenzoate in turn is obtained from the corresponding methyl ester of nitro-ortho-toluic acid by conventional bromination with N-bromosuccinimide under the influence of light.

Polymorphs of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione can be obtained by techniques known in the art, including solvent recrystallization, desolvation, vapor diffusion, rapid evaporation, slow evaporation, rapid cooling and slow cooling. Polymorphs can be made by dissolving a weighed quantity of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in various solvents at elevated temperatures. The solutions of the compound can then be filtered and allowed to evaporate either in an open vial (for fast hot evaporation) or in a vial covered with aluminum foil containing pinholes (hot slow evaporation). Polymorphs can also be obtained from slurries. Polymorphs can be crystallized from solutions or slurries using several methods. For example, a solution created at an elevated temperature (e.g., 60° C.) can be filtered quickly then allowed to cool to room temperature. Once at room temperature, the sample that did not crystallize can be moved to a refrigerator then filtered. Alternatively, the solutions can be crash cooled by dissolving the solid in a solvent at an increased temperature (e.g., 45-65° C.) followed by cooling in a dry ice/solvent bath.

One embodiment of the invention encompasses Form A of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form A is an unsolvated, crystalline material that can be obtained from non-aqueous solvent systems. Another embodiment of the invention encompasses Form B of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form B is a hemihydrated, crystalline material that can be obtained from various solvent systems. Another embodiment of the invention encompasses Form C of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form C is a hemisolvated crystalline material that can be obtained from solvents such as, but not limited to, acetone. Another embodiment of the invention encompasses Form D of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form D is a crystalline, solvated polymorph prepared from a mixture of acetonitrile and water. Another embodiment of the invention encompasses Form E of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form E is a dihydrated, crystalline material. Another embodiment of the invention encompasses Form F of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form F is an unsolvated, crystalline material that can be obtained from the dehydration of Form E. Another embodiment of the invention encompasses Form G of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form G is an unsolvated, crystalline material that can be obtained from slurrying forms B and E in a solvent such as, but not limited to, tetrahydrofuran (THF). Another embodiment of the invention encompasses Form H of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Form H is a partially hydrated crystalline material that can be obtained by exposing Form E to 0% relative humidity. Each of these forms is discussed in detail below.

Another embodiment of the invention encompasses a composition comprising amorphous 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione and crystalline 3-(4-

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amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione of form A, B, C, D, E, F, G or H. Specific compositions can comprise greater than about 50, 75, 90 or 95 weight percent crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Another embodiment of the invention encompasses a composition comprising at least two crystalline forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione (e.g., a mixture of polymorph forms B and E).

## 5.2.1 Form A

The data described herein for Form A, as well as for Forms B-H, were obtained using the experimental methods described in Examples 6.3-6.7, provided below.

Form A can be obtained from various solvents, including, but not limited to 1-butanol, butyl acetate, ethanol, ethyl acetate, methanol, methyl ethyl ketone, and THF. FIG. 1 shows a representative XRPD pattern of Form A. The pattern is characterized by peaks, preferably significant peaks, at approximately 8, 14.5, 16, 17.5, 20.5, 24, and 26 degrees 2θ. Representative IR and Raman spectra data are provided in FIGS. 2 and 3.

Representative thermal characteristics of Form A are shown in FIG. 4. TGA data show a small weight increase up to about 150° C., indicating an unsolvated material. Weight loss above 150° C. is attributed to decomposition. The DSC curve of Form A exhibits an endotherm at about 270° C.

Representative moisture sorption and desorption data are plotted in FIG. 5. Form A does not exhibit a significant weight gain from 5 to 95% relative humidity. Equilibrium can be obtained at each relative humidity step. As the form dries from 95% back down to 5% relative humidity, it tends to maintain its weight such that at 5% relative humidity it has typically lost only about 0.003% by weight from start to finish. Form A is capable of remaining a crystalline solid for about 11 days when stored at about 22, 45, 58, and 84% relative humidity.

Interconversion studies show that Form A can convert to Form B in aqueous solvent systems and can convert to Form C in acetone solvent systems. Form A tends to be stable in anhydrous solvent systems. In water systems and in the presence of Form E, Form A tends to convert to Form E.

When stored for a period of about 85 days under two different temperature/relative humidity stress conditions (room temperature/0% relative humidity (RH) and 40° C./93% RH), Form A typically does not convert to a different form.

In sum, Form A is a crystalline, unsolvated solid that melts at approximately 270° C. Form A is weakly or not hygroscopic and appears to be the most thermodynamically stable anhydrous polymorph of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione discovered thus far.

## 5.2.2 Form B

Form B can be obtained from many solvents, including, but not limited to, hexane, toluene, and water. FIG. 6 shows a representative XRPD pattern of Form B, characterized by peaks at approximately 16, 18, 22 and 27 degrees 2θ.

Solution proton NMR confirm that Form B is a form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Representative IR and Raman spectra are shown in FIGS. 7 and 8, respectively. Compared to Form A, the IR spectrum for Form B has peaks at approximately 3513 and 1960 cm<sup>-1</sup>.

Representative DSC and TGA data for Form B are shown in FIG. 9. The DSC curve exhibits endotherms at about 146 and 268° C. These events are identified as dehydration and melting by hot stage microscopy experiments. Form B typically loses about 3.1% volatiles up to about 175° C. (per



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approximately 0.46 moles of water). Comparison of the IR spectrum of the volatiles with that of water indicates that they are water (See FIG. 10). Calculations from TGA data indicate that Form B is a hemihydrate. Karl Fischer water analysis also supports this conclusion.

Representative moisture sorption and desorption data are shown in FIG. 11. Form B typically does not exhibit a significant weight gain from 5% to 95% relative humidity, when equilibrium is obtained at each relative humidity step. As Form B dries from 95% back down to 5% relative humidity, it tends to maintain its weight such that at 5% relative humidity it typically has gained only about 0.022% by weight (about 0.003 mg) from start to finish. Form B does not convert to a different form upon exposure to about 84% relative humidity for about ten days.

Interconversion studies show that Form B typically converts to Form A in a THF solvent system, and typically converts to Form C in an acetone solvent system. In aqueous solvent systems such as pure water and 10% water solutions, Form B is the most stable of the polymorphic forms of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. However, it can convert to Form E in the presence of water. Desolvation experiments show that upon heating at about 175° C. for about five minutes, Form B typically converts to Form A.

When stored for a period of about 85 days under two different temperature/relative humidity stress conditions (room temperature/0% RH and 40° C./93% RH), Form B does not convert to a different form.

In sum, Form B is a hemihydrated, crystalline solid which has a DSC thermogram exhibiting endotherms at about 146 and about 268° C. Interconversion studies show that Form B converts to Form E in aqueous solvent systems, and converts to other forms in acetone and other anhydrous systems.

#### 5.2.3 Form C

Form C can be obtained from evaporations, slurries and slow cools in acetone solvent systems. A representative XRPD pattern of this form is shown in FIG. 12. The data are characterized by peaks at approximately 15.5 and 25 degrees 2 $\theta$ .

Solution proton NMR indicates that the 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione molecule is intact. Representative IR and Raman spectra are shown in FIGS. 13 and 14, respectively. The IR spectrum of Form C is characterized by peaks at approximately 3466, 3373, and 3318 cm<sup>-1</sup>. The Raman spectrum of Form C is characterized by peaks at about 3366, 3321, 1101, and 595 cm<sup>-1</sup>.

Representative thermal characteristics for Form C are plotted in FIG. 15. Form C loses about 10.02% volatiles up to about 175° C., indicating it is a solvated material. Weight loss above about 175° C. is attributed to decomposition. Identification of volatiles in Form C can be accomplished with TG-IR experiments. The representative IR spectrum captured after several minutes of heating, as depicted in FIG. 13, when compared with a spectral library, shows acetone to be the best match. Calculations from TGA data show that Form C is a hemisolvate (approximately 0.497 moles of acetone). The DSC curve for Form C, shown in FIG. 15, exhibits endotherms at about 150 and about 269° C. The endotherm at about 150° C. is attributed to solvent loss based on observations made during hot stage microscopy experiments. The endotherm at about 269° C. is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption balance data are shown in FIG. 17. Form C does not exhibit a significant weight gain from 5 to 85% relative humidity, when equilibrium is obtained at each relative humidity step up to

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85% relative humidity. At 95% relative humidity, Form C experiences a significant weight loss of about 6.03%. As the sample dries from 95% back down to 5% relative humidity, the sample maintains the weight achieved at the end of the adsorption phase at each step down to 5% relative humidity. Form C is capable of converting to Form B when stored at about 84% relative humidity for approximately ten days.

Interconversion studies show that Form C typically converts to Form A in a THF solvent system and typically converts to Form E in an aqueous solvent system. In an acetone solvent system, Form C is the most stable form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Desolvation experiments performed on Form C show that upon heating at about 150° C. for about five minutes, Form C will typically convert to Form A.

In sum, Form C is a crystalline, hemisolvated solid, which has a DSC thermogram exhibiting endotherms at about 150 and about 269° C. Form C is not hygroscopic below about 85% RH, but can convert to Form B at higher relative humidities.

#### 5.2.4 Form D

Form D can be obtained from evaporation in acetonitrile solvent systems. A representative XRPD pattern of the form is shown in FIG. 18. The pattern is characterized by peaks at approximately 27 and 28 degrees 2 $\theta$ .

Solution proton NMR indicates that the 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione molecule is intact. Representative IR and Raman spectra are shown in FIGS. 19 and 20, respectively. The IR spectrum of Form D is characterized by peaks at approximately 3509, 2299, and 2256 cm<sup>-1</sup>. The Raman spectrum of Form D is characterized by peaks at approximately 2943, 2889, 2297, 2260, 1646, and 1150 cm<sup>-1</sup>.

Representative thermal characteristics for Form D are plotted in FIG. 21. Form D loses about 6.75% volatiles up to about 175° C., indicating a solvated material. Weight loss above about 175° C. is attributed to decomposition. TG-IR experiments indicate that the volatiles are water and acetonitrile. Calculations from TG data show that about one mole of water is present in the sample. A representative DSC curve for Form D exhibits endotherms at about 122 and about 270° C. The endotherm at about 122° C. is attributed to loss of volatiles based on observations made during hot stage microscopy experiments. The endotherm at about 270° C. is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption data are plotted in FIG. 22. Form D does not exhibit a significant weight gain from 5 to 95% relative humidity when equilibrium is obtained at each relative humidity step. As the form dries from 95% back down to 5% relative humidity, it maintains its weight such that at 5% relative humidity the form has typically gained only about 0.39% by weight (about 0.012 mg) from start to finish. Form A is capable of converting to Form B when stored at about 84% relative humidity for approximately ten days.

Interconversion studies show that Form D is capable of converting to Form A in a THF solvent system, to Form E in an aqueous solvent system, and to Form C in an acetone solvent system. Desolvation experiments performed on Form D show that upon heating at about 150° C. for about five minutes Form D will typically convert to Form A.

In sum, Form D is a crystalline solid, solvated with both water and acetonitrile, which has a DSC thermogram exhibiting endotherms at about 122 and about 270° C. Form D is either weakly or not hygroscopic, but will typically convert to Form B when stressed at higher relative humidities.

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## 5.2.5 Form E

Form E can be obtained by slurrying 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in water and by a slow evaporation of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione in a solvent system with a ratio of about 9:1 acetone:water. A representative XRPD pattern is shown in FIG. 23. The data are characterized by peaks at approximately 20, 24.5 and 29 degrees 2 $\theta$ .

Representative thermal characteristics of Form E are plotted in FIG. 24. Form E typically loses about 10.58% volatiles up to about 125° C., indicating that it is a solvated material. A second weight loss of an additional about 1.38% was observed between about 125° C. and about 175° C. Weight loss above about 175° C. is attributed to decomposition. Karl Fischer and TG-IR experiments support the conclusion that the volatile weight loss in Form E is due to water. The representative DSC curve for Form E exhibits endotherms at about 99, 161 and 269° C. Based on observations made during hot stage microscopy experiments, the endotherms at about 99 and about 161° C. are attributed to loss of volatiles. The endotherm at about 269° C. is attributed to the melt based on hot stage experiments.

Representative moisture sorption and desorption data are plotted in FIG. 25. Form E typically does not exhibit a significant weight change from 5 to 95% relative humidity when equilibrium is obtained at each relative humidity step. As the sample dried from 95% back down to 5% relative humidity, the sample continues to maintain weight such that at 5% relative humidity the sample has lost only about 0.0528% by weight from start to finish.

Interconversion studies show that Form E can convert to Form C in an acetone solvent system and to Form G in a THF solvent system. In aqueous solvent systems, Form E appears to be the most stable form. Desolvation experiments performed on Form E show that upon heating at about 125° C. for about five minutes, Form E can convert to Form B. Upon heating at 175° C. for about five minutes, Form B can convert to Form F.

When stored for a period of 85 days under two different temperature/relative humidity stress conditions (room temperature/0% RH and 40° C./93% RH) Form E typically does not convert to a different form. When stored for seven days at room temperature/0% RH, Form E can convert to a new form, Form H.

## 5.2.6 Form F

Form F can be obtained by complete dehydration of Form E. A representative XRPD pattern of Form F, shown in FIG. 26, is characterized by peaks at approximately 19, 19.5 and 25 degrees 2 $\theta$ .

Representative thermal characteristics of Form F are shown in FIG. 27. The representative DSC curve for Form F exhibits an endotherm at about 269° C. preceded directly by two smaller endotherms indicative of a crystallized form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The DSC thermogram does not show any thermal events prior to the melt, suggesting that it is an unsolvated material.

## 5.2.7 Form G

Form G can be obtained by slurrying forms B and E in THF. A representative XRPD pattern of this form, shown in FIG. 28, is characterized by a peak at approximately 23 degrees 2 $\theta$ . Two other peaks unique to Form G appear at approximately 21 and 24.5 degrees 2 $\theta$ .

Representative thermal characteristics of Form G are plotted in FIG. 29. A representative DSC curve for Form G exhibits an endotherm at about 248° C. followed by a small, broad exotherm at about 267° C. No thermal events are seen

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in the DSC thermogram at lower temperatures, suggesting that it is an unsolvated material.

## 5.2.8 Form H

Form H can be obtained by storing Form E at room temperature and 0% RH for about 7 days. A representative XRPD pattern is shown in FIG. 30. The pattern is characterized by a peak at 15 degrees 2 $\theta$ , and two other peaks at 26 and 31 degrees 2 $\theta$ .

Representative thermal characteristics are shown in FIG. 31. Form H loses about 1.67% volatiles up to about 150° C. Weight loss above about 150° C. is attributed to decomposition. Karl Fischer data shows that Form H typically contains about 1.77% water (about 0.26 moles), suggesting that the weight loss seen in the TG is due to dehydration. The DSC thermogram shows a broad endotherm between about 50° C. and about 125° C., corresponding to the dehydration of Form H and a sharp endotherm at about 269° C., which is likely due to a melt.

When slurried in water with either Forms A or B, after about 14 days Form H can convert to Form E. When slurried in THF, Form H can convert to Form A. When slurried in acetone, Form H can convert to Form C.

In sum, Form H is a crystalline solid, hydrated with about 0.25 moles of water, which has a DSC thermogram exhibiting an endotherm between about 50 and 125° C. and an endotherm at about 269° C.

## 5.3 Methods of Use and Pharmaceutical Compositions

Polymorphs of the invention exhibit physical characteristics that are beneficial for drug manufacture, storage or use. All polymorphs of the invention have utility as pharmaceutically active ingredients or intermediates thereof.

This invention encompasses methods of treating and preventing a wide variety of diseases and conditions using polymorphs of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. In each of the methods, a therapeutically or prophylactically effective amount of the compound is administered to a patient in need of such treatment or prevention. Examples of such disease and conditions include, but are not limited to, diseases associated with undesired angiogenesis, cancer (e.g., solid and blood borne tumors), inflammatory diseases, autoimmune diseases, and immune diseases. Examples of cancers and pre-cancerous conditions include those described in U.S. Pat. Nos. 6,281,230 and 5,635,517 to Muller et al. and in various U.S. patent applications to Zeldis, including application Ser. Nos. 10/411,649, filed Apr. 11, 2003 (Treatment of Myelodysplastic Syndrome); 10/438,213 filed May 15, 2003 (Treatment of Various Types of Cancer); 10/411,656, filed Apr. 11, 2003 (Treatment of Myeloproliferative Diseases). Examples of other diseases and disorders that can be treated or prevented using compositions of the invention are described in U.S. Pat. Nos. 6,235,756 and 6,114,335 to D'Amato and in other U.S. patent applications to Zeldis, including Ser. Nos. 10/693,794, filed Oct. 23, 2003 (Treatment of Pain Syndrome) and 10/699,154, filed Oct. 30, 2003 (Treatment of Macular Degeneration). The entirety of each of the patents and patent applications cited herein is incorporated herein by reference.

Depending on the disease to be treated and the subject's condition, polymorphs of the invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implantation), inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes of administration and may be formulated, alone or together, in suitable dosage unit

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formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration. Because individual polymorphs have different dissolution, stability, and other properties, the optimal polymorph used in methods of treatment may depend on the route of administration. For example, forms that are readily soluble in aqueous solutions are preferably used to provide liquid dosage forms, whereas forms that exhibit great thermal stability may be preferred in the manufacture of solid dosage forms (e.g., tablets and capsules).

Although the physical characteristics of polymorphs can, in some cases, affect their bioavailability, amounts of the polymorphs that are therapeutically or prophylactically effective in the treatment of various disease and conditions can be readily determined by those of ordinary skill in the pharmacy or medical arts. In certain embodiments of the invention, a polymorph is administered orally and in a single or divided daily doses in an amount of from about 0.10 to about 150 mg/day, or from about 5 to about 25 mg/day. In other embodiments, a polymorph is administered every other day in an amount of from about 0.10 to about 150 mg/day, or from about 5 to about 25 mg/day.

The invention encompasses pharmaceutical compositions and single unit dosage forms that can be used in methods of treatment and prevention, which comprise one or more polymorphs of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione and optionally one or more excipients or diluents. Specific compositions and dosage forms are disclosed in the various patents and patent applications incorporated herein by reference. In one embodiment, a single dosage form comprises a polymorph (e.g., Form B) in an amount of about 5, 10, 25 or 50 mg.

## 6. EXAMPLES

### 6.1 Polymorph Screen

A polymorph screen to generate the different solid forms of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione was carried out as follows.

A weighed sample of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (usually about 10 mg) was treated with aliquots of the test solvent. Solvents were either reagent or HPLC grade. The aliquots were usually about 200  $\mu$ L. Between additions, the mixture was usually shaken or sonicated. When the solids dissolved, as judged by visual inspection, estimated solubilities were calculated. Solubilities were estimated from these experiments based on the total solvent used to provide a solution. Actual solubilities may have been greater than those calculated due to the use of too-large solvent aliquots or to a slow rate of dissolution.

Samples were created by generating solutions (usually about 30 mg in 20 mL) at elevated temperatures, filtering, and allowing the solution to evaporate whether in an open vial (hot fast evaporation) or in a vial covered with aluminum foil containing pinholes (hot slow evaporation).

Slurry experiments were also performed. Usually about 25 mg of solid was placed in either 3 or 5 mL of solvent. The samples were then placed on orbital shakers at either ambient temperature or 40° C. for 410 days.

Crystallizations were performed using various cooling methods. Solid was dissolved in a solvent at an elevated temperature (e.g., about 60° C.), filtered quickly and allowed to cool to room temperature. Once at room temperature, samples that did not crystallize were moved to a refrigerator. Solids were removed by filtration or decantation and allowed

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to dry in the air. Crash cools were performed by dissolving solid in a solvent at an increased temperature (e.g., about 45-65° C.) followed by cooling in a dry ice/acetone bath.

Hygroscopicity studies were performed by placing portions of each polymorph in an 84% relative humidity chamber for approximately one week.

Desolvation studies were carried out by heating each polymorph in a 70° C. oven for approximately one week.

Interconversion experiments were carried out by making slurries containing two forms in a saturated solvent. The slurries were agitated for approximately 7-20 days at ambient temperature. The insoluble solids were recovered by filtration and analyzed using XRPD.

### 6.2 Preparation of Polymorphic Forms

Eight solid forms of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione were prepared as described below.

Form A was obtained by crystallization from various non-aqueous solvents including 1-butanol, butyl acetate, ethanol, ethyl acetate, methanol, methyl ethyl ketone, and tetrahydrofuran. Form B was also obtained by crystallization from the solvents hexane, toluene and water. Form C was obtained from evaporations, slurries, and slow cools in acetone solvent systems. Form D was obtained from evaporations in acetonitrile solvent systems. Form E was obtained most readily by slurrying 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in water. Form F was obtained by complete desolvation of Form E. It is found to be an unsolvated, crystalline material that melts at about 269° C. Form G was obtained by slurrying forms B and E in THF. Form H was obtained by stressing Form E at room temperature and 0% RH for 7 days.

#### 6.2.1 Synthesis of Polymorphs B and E

Form B is the desired polymorph for the active pharmaceutical ingredient (API) of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. This form has been used in the formulation of API into drug product for clinical studies. Three batches were produced as apparent mixtures of polymorphs in the non-micronized API of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Development work was carried out to define a process that would generate polymorph B from this mixture of polymorphs and could be implemented for strict polymorphic controls in the validation batches and future manufacturing of API of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Characterization of polymorphic forms produced during the work was performed by XRPD, DSC, TGA and KF.

A process was also developed for the large-scale preparation of Form E. Polymorph E material was prepared in order to carry out a comparison with polymorph B drug product in capsule dissolution testing of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. 150 g of a mixture of polymorphs in 3 L of water was stirred at room temperature for 48 hours. The product was collected by filtration and dried at 25° C. for 24 hours under vacuum. XRPD, DSC, TGA, KF and HPLC analyses confirmed that the material isolated was polymorph E.

In a preliminary work, it was demonstrated that stirring a suspension of a mixture of polymorphs of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione with water at high temperature (75° C.) for an extended period of time converted this mixture of polymorphs exclusively to form B. Several specific parameters were identified including temperature, solvent volume and drying parameters (temperature



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and vacuum). XRPD, DSC, TGA, KF and HPLC analyses were used to characterize all of the batches. After completing the optimization work, the optimized process was scaled-up to 100-200 g on three lots of API. Drying studies were carried out at 20° C., 30° C. and 40° C., and 65° C. with a vacuum of 150 mm of Hg. The results are shown in Tables 1-5.

The cooling and holding periods of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione slurry were studied. The experimental laboratory data suggests that polymorph B seems to be forming first, and overtime equilibration to polymorph E at RT conditions occurs, therefore generating a mixture of polymorphs B and E. This result supports the fact that polymorph B seems to be a kinetic product, and that prolonged processing time converts the material to polymorph E resulting in a mixture of polymorphs B and E.

A laboratory procedure was developed to exclusively produce polymorph B of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. The procedure includes a stirred 10 volume water slurry at ~75° C. for 6-24 hours. The following preferred process parameters have been identified:

1. Hot slurry temperature of 70-75° C.
2. Product filtration of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)piperidine-2,6-dione at 65-75° C.
3. Drying under vacuum at 60-70° C. is preferred for an efficient removal of unbound water in 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione wet cake.
4. The filtration step of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione may be a time sensitive operation. The use of efficient solid-liquid separation equipment is preferred.
5. Holding periods of water-wet cake of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione at KF higher than 5% may cause the kinetic equilibrations of polymorph B to mixed polymorphs of E and B.

Drying to KF<4.0% water was achieved in ~3 hours (30-70° C., 152 mm Hg). Polymorphs B and E were distinguished by the water levels as measured by KF and TGA. The reference sample of polymorph B is micronized API. In order to make accurate comparison by XRPD samples were gently grinded before submission for analysis. This increases the clarity of the identification of the polymorphic form. All samples were analyzed for XRPD, DSC, TGA, KF and HPLC.

TABLE 1

Preliminary Studies			
Amount	Reaction conditions	Analysis	Results/conclusion
2 g	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
25 g	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
5 g	Water, 70-75° C., 24 h then rt 24 h	XRPD, DSC, TGA, KF	Polymorph B
1 g	9:1 Acetone - water, Slow evpo.	XRPD, DSC, TGA, KF	Polymorph Mixture
1 g	175° C. 1 h in an oven	XRPD, DSC, TGA, KF	Polymorph A
0.5 g (polymorph A)	Water, rt, 24 h	XRPD, DSC, TGA, KF	Polymorph E
1 g polymorph B	Water, rt, 48 h	XRPD, DSC, TGA, KF	Polymorph E
1 g polymorph E	Water, 70-75° C., 24 h	XRPD, DSC, TGA, KF	Polymorph B
1 g	Slurry in heptane	XRPD, DSC, TGA, KF	No change

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TABLE 2

Optimization of Temperature, Time and Solvent Volume				
Amount	Amount Water (mL)	Temp (° C.)	Time (h)	Results/conclusion
10 g	50	75	6	Mix
10 g	50	75	24	Polymorph B
10 g	100	70	6	Polymorph B
10 g	100	70	14	Polymorph B
10 g	100	70	21	Polymorph B
10 g	100	75	6	Polymorph B
10 g	100	75	24	Polymorph B
10 g	100	75	6	Polymorph B
10 g	100	75	19	Polymorph B
10 g	100	75	14	Polymorph B
10 g	100	75	24	Polymorph B
5 g	100	75	18	Polymorph B
10 g	100	80	6	Polymorph B
10 g	100	80	20	Polymorph B
10 g	200	45	6	Polymorph B + E
10 g	200	45	24	Polymorph E
10 g	200	60	48	Polymorph B
10 g	200	75	6	Mix
10 g	200	75	24	Polymorph B
10 g	200	75	13	Polymorph B
10 g	200	75	24	Polymorph B

Optimum conditions were determined to be 10 volumes of solvent (H<sub>2</sub>O), 70-80° C. for 6-24 hours.

TABLE 3

Holding Time				
Amount	Reaction Conditions	Holding Time (h)	Holding Temp (° C.)	Results/Conclusion
5 g	Water, 70-75° C., 24 h	24	23-25	Polymorph B
1 g	Water, 70-75° C., 24 h	48	23-25	Polymorph E
Polymorph B				
2 g	Water, 40 mL	16	23-25	Polymorph E
150 g	Water, 3.0 L	24	23-25	Polymorph E
150 g	Water, 3.0 L	48	23-25	Polymorph E
10 g	Water, 100 mL, 24 h, 75° C.	18	23-25	Polymorph B
10 g	Water, 100 mL, 24 h, 75° C.	18	40	Polymorph B
10 g	Water, 200 mL, 24 h, 75° C.	14	-5	Mix
10 g	Water, 200 mL, 24 h, 75° C.	14	23-25	Polymorph E
10 g	Water, 200 mL, 24 h, 75° C.	14	40	Mix
10 g	Water, 100 mL, 24 h, 75° C.	21	23-25	Polymorph E
10 g	Water, 100 mL, 24 h, 75° C.	21	40	Mix
10 g	Water, 100 mL, 14 h, 75° C.	2	23-25	Mix

Holding time gave mixed results and it was determined that the material should be filtered at 60-65° C. and the material washed with 0.5 volume of warm (50-60° C.) water.

TABLE 4

Scale-up Experiments				
Amount	Amount Water (L)	Temp (° C.)	Time (h)	Results/Conclusion
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	22	Polymorph B

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TABLE 4-continued

Scale-up Experiments				
Amount	Amount Water (L)	Temp (° C.)	Time (h)	Results/Conclusion
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	24	Polymorph B
100 g	1.0	75	6	Polymorph B
100 g	1.0	75	22	Polymorph B

TABLE 5

Drying Studies					
Amount	Drying Time (h)	Drying Temp (° C.)	Vacuum (mm Hg)	KF§ (%)	Results/Conclusion
100 g	0	—	—	3.690	Polymorph B
100 g	3	30	152	3.452	Polymorph B
100 g	8	30	152	3.599	Polymorph B
100 g	0	—	—	3.917	Polymorph B
100 g	5	40	152	3.482	Polymorph B
100 g	22	40	152	3.516	Polymorph B
100 g	3	40	152	3.67	Polymorph B
100 g	22	40	152	3.55	Polymorph B

\*Reaction Conditions: Water 1 L, 75° C., 22-24 h;

§Average of 2 runs.

Drying studies determined that the material should be dried at 35-40° C., 125-152 mm Hg for 3 to 22 h or until the water content reaches  $\leq 4\%$  w/w.

For a large scale preparation of polymorph E (5222-152-B), a 5-L round bottom flask was charged with 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione (150 g, 0.579 mol) and water (3000 mL, 20 vol). The mixture was mechanically stirred at room temperature (23-25° C.) for 48 h under nitrogen atmosphere.

Samples were taken after 24 h and 48 h before the mixture was filtered and air-dried on the filter for 1 h. The material was transferred to a drying tray and dried at room temperature (23-25° C.) for 24 h. KF analysis on the dried material showed water content of 11.9%. The material was submitted for XRPD, TGA, DSC and HPLC analysis. Analysis showed the material was pure polymorph E.

For a large scale preparation of polymorph B (5274-104), a 2 L-3-necked round bottom flask was charged with 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione (polymorph mixture, 100 g, 0.386 mol) and water (1000 mL, 10.0 vol). The mixture was heated to 75° C. over approximately 30 minutes with mechanical stirring under nitrogen atmosphere.

Samples were taken after 6 h and 24 h before the mixture was allowed to cool to 60-65° C., filtered and the material washed with warm (50-60° C.) water (50 mL, 0.5 vol). The material was transferred to a drying tray and dried at 30° C., 152 mm Hg for 8 h. KF analysis on the dried material showed water content of 3.6%. After grinding the material was submitted for XRPD, TGA, DSC and HPLC analysis. Analysis showed the material was pure polymorph B. The results of the analyses are shown in FIGS. 32-46.

## 6.3 X-Ray Powder Diffraction Measurements

X-ray powder diffraction analyses were carried out on a Shimadzu XRD 6000 X-ray powder diffractometer using Cu K $\alpha$  radiation. The instrument is equipped with a fine-focus X-ray tube. The tube voltage and amperage were set at 40 kV

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and 40 mA, respectively. The divergence and scattering slits were set at 1° and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3°/min (0.4 sec/0.02° step) from 2.5 degrees 2 $\theta$  to 40 degrees 2 $\theta$  was used. A silicon standard was analyzed each day to check the instrument alignment.

X-ray powder diffraction analyses were also carried out using Cu K $\alpha$  radiation on an Inel XRG-3000 diffractometer equipped with a curved position-sensitive detector. Data were collected in real time over a theta-two theta range of 120° at a resolution of 0.03°. The tube voltage and current were 40 kV and 30 mA, respectively. A silicon standard was analyzed each day to check for instrument alignment. Only the region between 2.5 and 40 degrees 2 $\theta$  is shown in the figures.

## 6.4 Thermal Analysis

TG analyses were carried out on a TA Instrument TGA 2050 or 2950. The calibration standards were nickel and alumel. Approximately 5 mg of sample was placed on a pan, accurately weighed, and inserted into the TG furnace. The samples were heated in nitrogen at a rate of 10° C./min, up to a final temperature of 300 or 350° C.

DSC data were obtained on a TA 2920 instrument. The calibration standard was indium. Approximately 2-5 mg samples were placed into a DSC pan and the weight accurately recorded. Crimped pans with one pinhole were used for analysis and the samples were heated under nitrogen at a rate of 10° C./min, up to a final temperature of 350° C.

Hot-stage microscopy was carried out using a Kofler hot stage mounted on a Leica Microscope. The instrument was calibrated using USP standards.

A TA Instruments TGA 2050 interfaced with a Nicolet model 560 Fourier transform IR spectrophotometer, equipped with a globar source, XT/KBr beamsplitter, and deuterated triglycine sulfate (DTGS) detector, was utilized for TG-IR experiments. The IR spectrometer was wavelength calibrated with polystyrene on the day of use while the TG was temperature and weight calibrated biweekly, using indium for the temperature calibration. A sample of approximately 10 mg of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione was weighed into an aluminum pan and heated from 25 to 30° C. to 200° C. at a rate of 20° C./min with a helium purge. IR spectra were obtained in series, with each spectrum representing 32 co-added scans at a resolution of 4 cm<sup>-1</sup>. Spectra were collected with a 17-second repeat time. TG/IR analysis data are presented as Gram-Schmidt plots and IR spectra linked to the time. Gram-Schmidt plots show total IR intensity vs. time; hence, the volatiles can be identified at each time point. They also show when the volatiles are detected. From the Gram-Schmidt plots, time points were selected and the IR spectra of these time points are presented in the stacked linked spectra. Each spectrum identifies volatiles evolving at that time point. Volatiles were identified from a search of the HR Nicolet TGA vapor phase spectral library. The library match results are also presented to show the identified vapor.

## 6.5 Spectroscopy Measurements

Raman spectra were acquired on a Nicolet model 750 Fourier transform Raman spectrometer utilizing an excitation wavelength of 1064 nm and approximately 0.5 W of Nd:YAG laser power. The spectra represent 128 to 256 co-added scans acquired at 4 cm<sup>-1</sup> resolution. The samples were prepared for analysis by placing the material in a sample holder and posi-

tioning this in the spectrometer. The spectrometer was wavelength calibrated using sulfur and cyclohexane at the time of use.

The mid-IR spectra were acquired on a Nicolet model 860 Fourier transform IR spectrophotometer equipped with a global source XT/KBr beamsplitter and a deuterated triglycine sulfate (DTGS) detector. A Spectra-Tech, Inc. diffuse reflectance accessory was utilized for sampling. Each spectrum represents 128 co-added scans at a spectral resolution of 4  $\text{cm}^{-1}$ . A background data set was acquired with an alignment mirror in place. A single beam sample data set was then acquired. Subsequently, a log 1/R (where R=reflectance) spectrum was acquired by rationing the two data sets against each other. The spectrophotometer was calibrated (wavelength) with polystyrene at the time of use.

#### 6.6 Moisture Sorption/Desorption Measurements

Moisture sorption/desorption data were collected on a VTI SGA-100 moisture balance system. For sorption isotherms, a sorption range of 5 to 95% relative humidity (RH) and a desorption range of 95 to 5% RH in 10% RH increments was used for analysis. The sample was not dried prior to analysis. Equilibrium criteria used for analysis were less than 0.0100 weight percent change in 5 minutes with a maximum equilibration time of 3 hours if the weight criterion was not met. Data were not corrected for the initial moisture content of the samples.

#### 6.7 Solution Proton NMR Measurements

NMR spectra not previously reported were collected at SSCI, Inc, 3065 Kent Avenue, West Lafayette, Ind. Solution phase  $^1\text{H}$  NMR spectra were acquired at ambient temperature on a Bruker model AM spectrometer. The  $^1\text{H}$  NMR spectrum represents 128 co added transients collected with a 4  $\mu\text{sec}$  pulse and a relaxation delay time of 5 seconds. The free induction decay (FID) was exponentially multiplied with a 0.1 Hz Lorentzian line broadening factor to improve the signal-to-noise ratio. The NMR spectrum was processed utilizing GRAMS software, version 5.24. Samples were dissolved in dimethyl sulfoxide- $d_6$ .

The scope of this invention can be understood with reference to the appended claims.

#### 6.8 Intrinsic Dissolution and Solubility Studies

Intrinsic dissolution experiments were conducted on Form A (anhydrous), Form B (hemihydrate), and Form E (dihydrate) of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione. Equilibrium solubility experiments were conducted on Forms A and B. Aliquots were analyzed by ultraviolet-visible spectrophotometry, and the solids remaining from each experiment were analyzed by X-ray powder diffraction (XRPD).

##### 6.8.1 Experimental

##### 6.8.1.1 Dissolution

Dissolution experiments were carried out in a VanKel VK6010-8 dissolution apparatus equipped with a VK650A heater/circulator. An intrinsic dissolution apparatus (Woods apparatus) was used. Samples were compressed at 1.5 metric tons (1000 psi) for 1 min using the Woods apparatus in a hydraulic press, giving a sample surface of 0.50  $\text{cm}^2$ . A dissolution medium consisting of 900 mL HCl buffer, pH 1.8, with 1% sodium lauryl sulfate, was used for each experiment. The medium was degassed by vacuum filtration through a 0.22- $\mu\text{m}$  nylon filter disk and maintained at 37° C. The appa-

ratus was rotated at 50 rpm for each experiment. Aliquots were filtered immediately using 0.2- $\mu\text{m}$  nylon syringe filters. In some cases, the undissolved solids were recovered and analyzed by X-ray powder diffraction (XRPD).

##### 6.8.1.2 Solubility

Equilibrium solubility experiments were conducted in a 100-mL, three-neck, round-bottom flask immersed in a constant temperature oil bath maintained at 25° C. A solid sample of 400-450 mg was stirred in 50 mL of dissolution medium (HCl buffer, pH 1.8, with 1% sodium lauryl sulfate) using a mechanical stir rod. Aliquots were filtered using 0.2- $\mu\text{m}$  nylon syringe filters and immediately diluted 1 mL→50 mL, then 5 mL→25 mL with dissolution medium in Class A glassware, a final dilution factor of 250.

##### 6.8.1.3 UV-Vis Spectrophotometry

Dissolution and solubility samples solutions were analyzed by a Beckman DU 640 single-beam spectrophotometer. A 1.000-cm quartz cuvette and an analysis wavelength of 228.40 nm were utilized. The detector was zeroed with a cuvette filled with dissolution medium.

##### 6.8.1.4 X-Ray Powder Diffraction

XRPD analyses were carried out on a Shimadzu XRD-6000 X-ray powder diffractometer using Cu  $K\alpha$  radiation. The instrument is equipped with a fine focus X-ray tube. The tube power and amperage were set at 40 kV and 40 mA, respectively. The divergence and scattering slits were set at 1° and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3°/min (0.4 sec/0.02° step) from 2.5 to 40° 2 $\theta$  was used. A silicon standard was analyzed each day to check the instrument alignment. Samples were packed in an aluminum holder with silicon insert.

##### 6.8.2 Results

The results of these solubility and intrinsic studies are summarized in Table 6. Both the solubility and dissolution experiments were conducted in a medium of HCl buffer, pH 1.8, containing 1% sodium lauryl sulfate. Form A was found to be unstable in the medium, converting to Form E. The solubilities of Forms A, B, and E were estimated to be 6.2, 5.8, and 4.7 mg/mL, respectively. The dissolution rates of Forms A, B, and E were estimated to be 0.35, 0.34, and 0.23 mg/mL, respectively.

##### 6.8.2.1 UV-Vis Spectrophotometry Method Development

A UV-V is scan of the dissolution medium (blanked with an empty cuvette) was done to identify any interfering peaks. A small peak at 225 nm was present as shown in FIG. 47.

Solutions of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione at varying concentrations were analyzed by UV-V is spectrophotometry. A preliminary scan of a 1.0 mg/mL solution was done, with the instrument blanked with dissolution medium. The solution was highly absorbing and noisy from 200-280 nm, making dilution necessary.

A 0.04 mg/mL solution of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione was then scanned from 200-300 nm. The plot was still noisy between 200 and 230 nm as shown in FIG. 48. The sample was further diluted to 0.008 mg/mL. A wavelength scan of 200-350 nm for this sample showed a peak at 228.4 nm with no interference, as shown in FIG. 49. Therefore, a wavelength of 228.4 was chosen for analysis of the solubility and dissolution samples.

A six-point calibration curve was generated with standards of the following concentrations: 0.001 mg/mL, 0.002 mg/mL, 0.005 mg/mL, 0.010 mg/mL, 0.015 mg/mL, and 0.020 mg/mL (Notebook 569-90). A linearity coefficient of  $R^2=0.9999$  was obtained as shown in FIG. 50.

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## 6.8.2.2 Solubility

A sample consisting of 449.4 mg Form A was slurried in dissolution medium. Particle size was not controlled. Aliquots were taken at 7, 15, 30, 60, 90, and 150 min. The concentration reached 6.0 mg/mL by the first time point. The highest concentration reached was 6.2 mg/mL, at 30 min. From that point the concentration decreased, reaching 4.7 mg/mL at 150 min as in FIG. 51. The solids remaining at the final time point were analyzed by XRPD and found to be Form E as shown in Table 7. No peaks attributed to Form A can be seen in the pattern. Since the concentration did not plateau at 4.7 mg/mL, the solubility of Form E may be lower than that.

A sample consisting of 401.4 mg Form B was slurried in dissolution medium. Particle size was not controlled. Aliquots were taken at 7, 15, 30, 60, 90, 180, 420, and 650 min. Form B dissolved much more slowly than Form A, reaching 3.3 mg/mL in 90 min. The concentration stabilized at 5.6-5.7 mg/mL at the final three time points as in FIG. 52. The remaining solids were shown to be Form B as in Table 7, suggesting Form B has good stability in water.

A summary of the solubilities is given in Table 6. The amounts dissolved at each time point are shown in Tables 8 and 9.

TABLE 6

Summary of Results				
Form	Solubility	Intrinsic Dissolution #1	Intrinsic Dissolution #2	Average Intrinsic Dissolution Rate
Form A	6.2 mg/mL	0.35	0.22 <sup>a</sup>	0.29 <sup>a</sup>
Form B	5.8 mg/mL	0.35	0.32	0.34
Form E	4.7 mg/mL	0.21	0.25	0.23

<sup>a</sup>The Form A dissolution experiment #2 may have converted to Form E on the surface of the disk, skewing the average rate lower.

TABLE 7

Experimental Details		Final Form
Experiment		
Pressed Form A		A
Pressed Form B		B
Form A Solubility		E
Form B Solubility		B
Form A Dissolution		—
Form B Dissolution		A
Form E Dissolution		—
Form B Dissolution		B
Form E Dissolution		E
Form E Dissolution		—

TABLE 8

Form A Solubility	
Time Point (min)	Concentration (mg/mL)
7	6.00
15	6.11
30	6.16
60	6.10
90	5.46
150	4.73

20

TABLE 9

Form B Solubility	
Time Point (min)	Concentration (mg/mL)
7	1.63
15	2.14
30	2.33
60	2.94
90	3.34
180	5.67
420	5.76
650	5.61

## 6.8.2.3 Intrinsic Dissolution

Approximately 200 mg each of Forms A and B were compressed into disks in the Woods apparatus using 2 metric tons of pressure. The samples were subsequently scraped out, ground gently, and analyzed by XRPD. The study showed that compression and grinding does not cause a form change in either case. (See Table 7).

Two preliminary dissolution runs were performed. The disks fractured to some extent in both experiments, compromising the requirement of constant surface area.

The first experiment of intrinsic dissolution that strictly followed the USP chapter on intrinsic dissolution utilized approximately 150 mg each of Forms A and B. Seven aliquots, beginning at 5 min and ending at 90 min, were taken to maintain sink conditions. The experiment resulted in linear dissolution profiles, with a rate of 0.35 mg per cm<sup>2</sup> per minute for both forms. The Form E experiment was done later under the same conditions and added to the graph for comparison. (See FIG. 53). The Form E dissolution rate was 0.21 mg per cm<sup>2</sup> per minute, significantly lower than the dissolution rate of Forms A and B. This is in line with expectations based on the solubility data. The crystal form of the remaining solids did not change in any case.

The second experiment utilized approximately 250 mg each of Forms A and B. The Form E experiment (135 mg) was done later and added to the graph for comparison. (See FIG. 54). Nine aliquots were taken, beginning at 5 min and ending at 150 min. The dissolution rates were 0.22, 0.32, and 0.25 mg per cm<sup>2</sup> per minute, respectively, for Forms A, B, and E. The dissolution rate for Form A in this experiment was low, while the rates for Forms B and E were similar to those found in the first experiment. It is believed that in this case, a thin layer of the Form A sample disk may have converted to Form E upon exposure to water. This is supported by the evidence of rapid conversion of Form A to Form E in the solubility experiment. The diffraction pattern of the undissolved solids does not indicate a form change. However, the bulk of the sample disk is not exposed to water. Therefore, the true intrinsic dissolution rate of Form A is believed to be close to 0.35 mg per cm<sup>2</sup> per minute. An insufficient quantity of Form A was available to repeat the experiment.

A summary of the intrinsic dissolution rates is given in Table 6. The amounts dissolved at each time point are summarized in Tables 10 and 11.

TABLE 10

Intrinsic Dissolution Experiment #1 Results			
Time Point	Form A <sup>a</sup>	Form B <sup>a</sup>	Form E <sup>a</sup>
5 min	5.76	10.80 <sup>b</sup>	2.70
10 min	7.73	6.85	4.13



TABLE 10-continued

Intrinsic Dissolution Experiment #1 Results			
Time Point	Form A <sup>a</sup>	Form B <sup>a</sup>	Form E <sup>a</sup>
20 min	11.31	10.25	6.96
30 min	15.59	14.35	9.60
45 min	21.98	20.57	12.57
60 min	27.11	25.70	15.16
90 min	34.17	34.34	20.82

<sup>a</sup>Results are reported as Cumulative Amount Dissolved per Unit Area (mg/cm2)  
<sup>b</sup>This date point not included in graph since the value is higher than the next two data points.

TABLE 11

Intrinsic Dissolution Experiment #2 Results			
Time Point	Form A <sup>a</sup>	Form B <sup>a</sup>	Form E <sup>a</sup>
5 min	4.50	5.04	3.06
10 min	5.22	6.12	4.31
20 min	7.54	7.73	11.40
30 min	11.46	12.72	11.93
45 min	15.01	17.33	14.72
60 min	18.38	21.93	18.52
90 min	24.38	31.64	26.24
120 min	30.35	41.31	33.56
150 min	35.26	49.54	40.82

<sup>a</sup>Results are reported as Cumulative Amount Dissolved per Unit Area (mg/cm2)

6.9 Analyses of Mixtures of Polymorphs

This invention encompasses mixtures of different polymorphs. For example, an X-ray diffraction analysis of one production sample yielded a pattern that contained two small peaks seen at approximately 12.6° and 25.8° 2θ in addition to those representative of Form B. In order to determine the composition of that sample, the following steps were performed.

- 1) Matching of the new production pattern to known forms along with common pharmaceutical excipients and contaminants;
- 2) Cluster analysis of the additional peaks to identify if any unknown phase is mixed with the original Form B;
- 3) Harmonic analysis of the additional peaks to identify if any preferred orientation may be present or if any changes in the crystal habit may have occurred; and
- 4) Indexing of the unit cells for both Form B and the new production sample to identify any possible crystallographic relationships.

Based on these tests, which can be adapted for the analysis of any mixture of polymorphs, it was determined that the sample contained a mixture of polymorph forms B and E.

6.10 Dosage Form

Table 12 illustrates a batch formulation and single dosage formulation for a 25 mg single dose unit of a polymorphic form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione.

TABLE 12

Formulation for a 25 mg capsule			
Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Polymorphic Form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione	40.0%	25 mg	16.80 kg
Pregelatinized Corn Starch, NF	59.5%	37.2 mg	24.99 kg
Magnesium Stearate	0.5%	0.31 mg	0.21 kg
Total	100.0%	62.5 mg	42.00 kg

The pregelatinized corn starch (SPRESS B-820) and polymorphic form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione components are passed through a screen (i.e., a 710 μm screen) and then loaded into a Diffusion Mixer with a baffle insert and blended for about 15 minutes. The magnesium stearate is passed through a screen (i.e., a 210 μm screen) and added to the Diffusion Mixer. The blend is then encapsulated in capsules using a Dosator type capsule filling machine.

The entire scope of this invention is not limited by the specific examples described herein, but is more readily understood with reference to the appended claims.

What is claimed is:

- 1. A solid form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione comprising crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate, wherein the crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate is present at greater than about 80% by weight of the solid form.
- 2. A solid form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione comprising crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate, wherein the crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate is present at greater than about 90% by weight of the solid form.
- 3. A solid form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione comprising crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate, wherein the crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate is present at greater than about 95% by weight of the solid form.
- 4. A solid form of 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione comprising crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate, wherein the crystalline 3-(4-amino-1-oxo-1,3 dihydro-isoindol-2-yl)-piperidine-2,6-dione hemihydrate is present at greater than about 97% by weight of the solid form.
- 5. A pharmaceutical composition comprising a therapeutically effective amount of the solid form of claim 1, 2, 3, or 4, and a pharmaceutically acceptable excipient or carrier.
- 6. The pharmaceutical composition of claim 5, which is a single unit dosage form.
- 7. The pharmaceutical composition of claim 5, wherein the therapeutically effective amount is about 5 mg.
- 8. The pharmaceutical composition of claim 5, wherein the therapeutically effective amount is about 10 mg.
- 9. The pharmaceutical composition of claim 5, wherein the therapeutically effective amount is about 25 mg.
- 10. The pharmaceutical composition of claim 5, wherein the therapeutically effective amount is about 50 mg.

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 7,855,217 B2  
APPLICATION NO. : 12/335395  
DATED : December 21, 2010  
INVENTOR(S) : Jaworsky et al.

Page 1 of 11

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Please replace Drawing Sheets 15, 26, 31, 32, 33, 35, 36, 38, 39, and 40 (of 48) with the Replacement Drawing Sheets on the following pages of this Certificate of Correction:

Page 2 of 11: Replacement Drawing Sheet 15 of 48 (Fig. 15);

Page 3 of 11: Replacement Drawing Sheet 26 of 48 (Fig. 26);

Page 4 of 11: Replacement Drawing Sheet 31 of 48 (Fig. 31);

Page 5 of 11: Replacement Drawing Sheet 32 of 48 (Figs. 32 and 33);

Page 6 of 11: Replacement Drawing Sheet 33 of 48 (Figs. 34 and 35);

Page 7 of 11: Replacement Drawing Sheet 35 of 48 (Figs. 37 and 38);

Page 8 of 11: Replacement Drawing Sheet 36 of 48 (Figs. 39 and 40);

Page 9 of 11: Replacement Drawing Sheet 38 of 48 (Figs. 42 and 43);

Page 10 of 11: Replacement Drawing Sheet 39 of 48 (Figs. 44 and 45);

Page 11 of 11: Replacement Drawing Sheet 40 of 48 (Fig. 46).

Signed and Sealed this  
Third Day of May, 2011

A handwritten signature in black ink that reads "David J. Kappos". The signature is written in a cursive, slightly slanted style.

David J. Kappos  
*Director of the United States Patent and Trademark Office*

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TGA (TOP) AND DSC (BOTTOM) OF FORM C

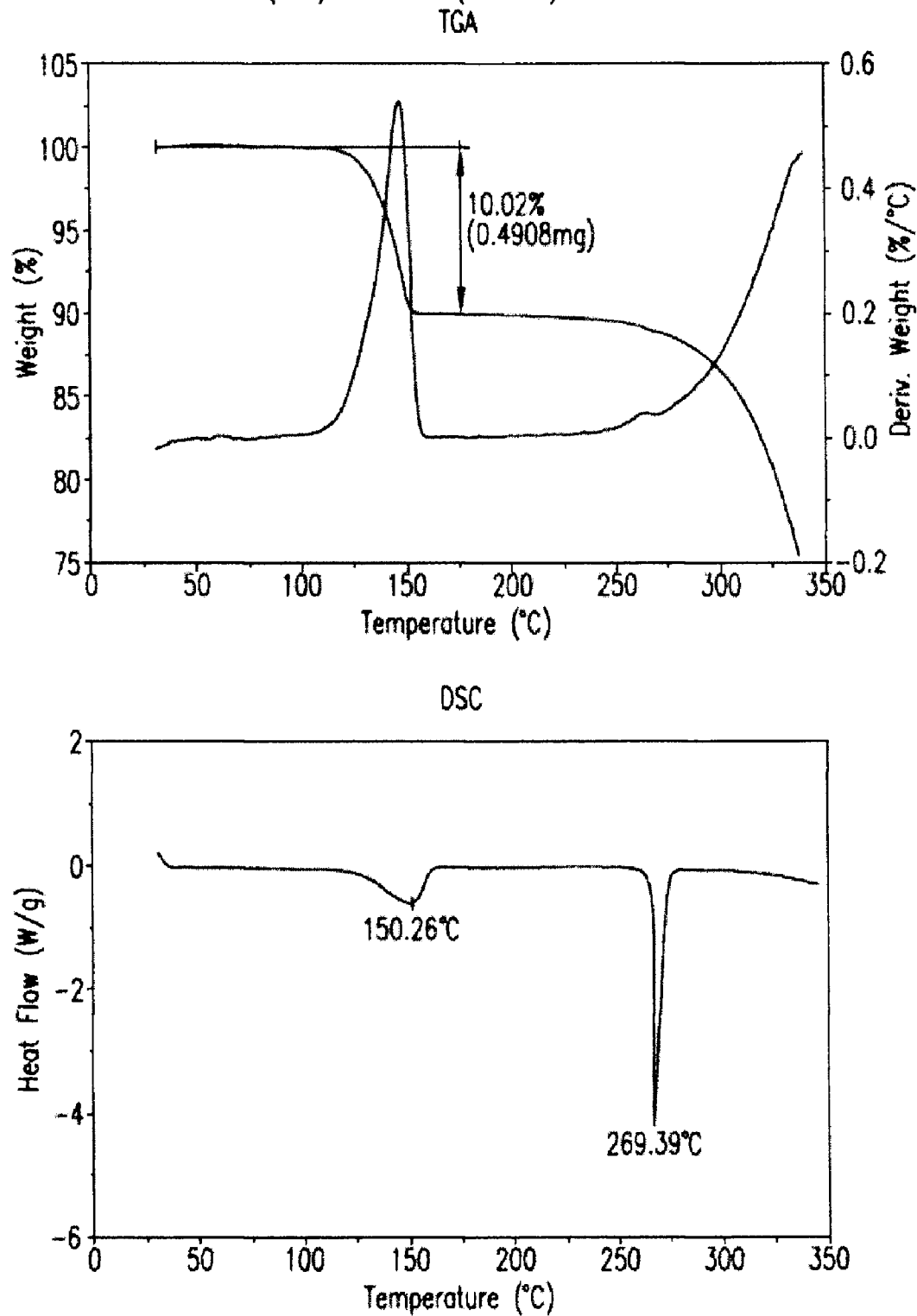


FIG.15

CERTIFICATE OF CORRECTION (continued)

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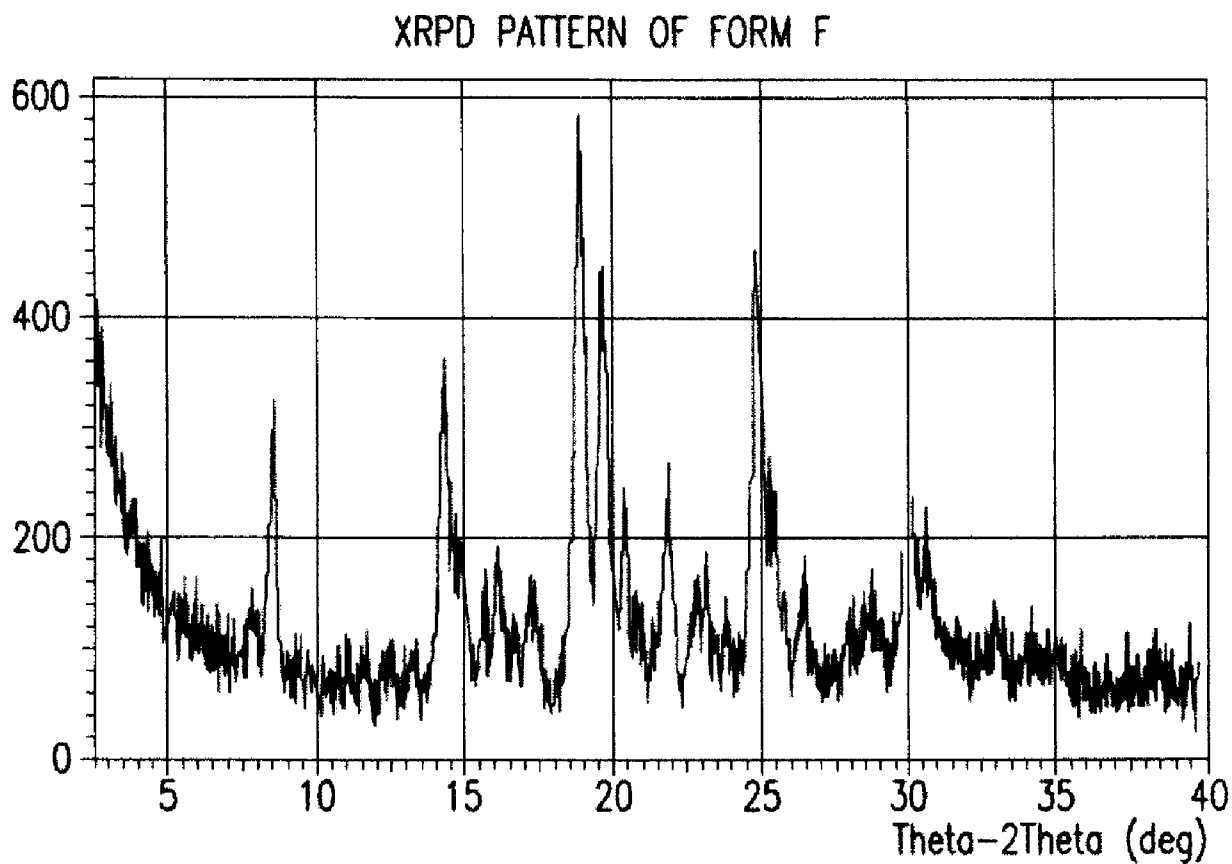


FIG.26



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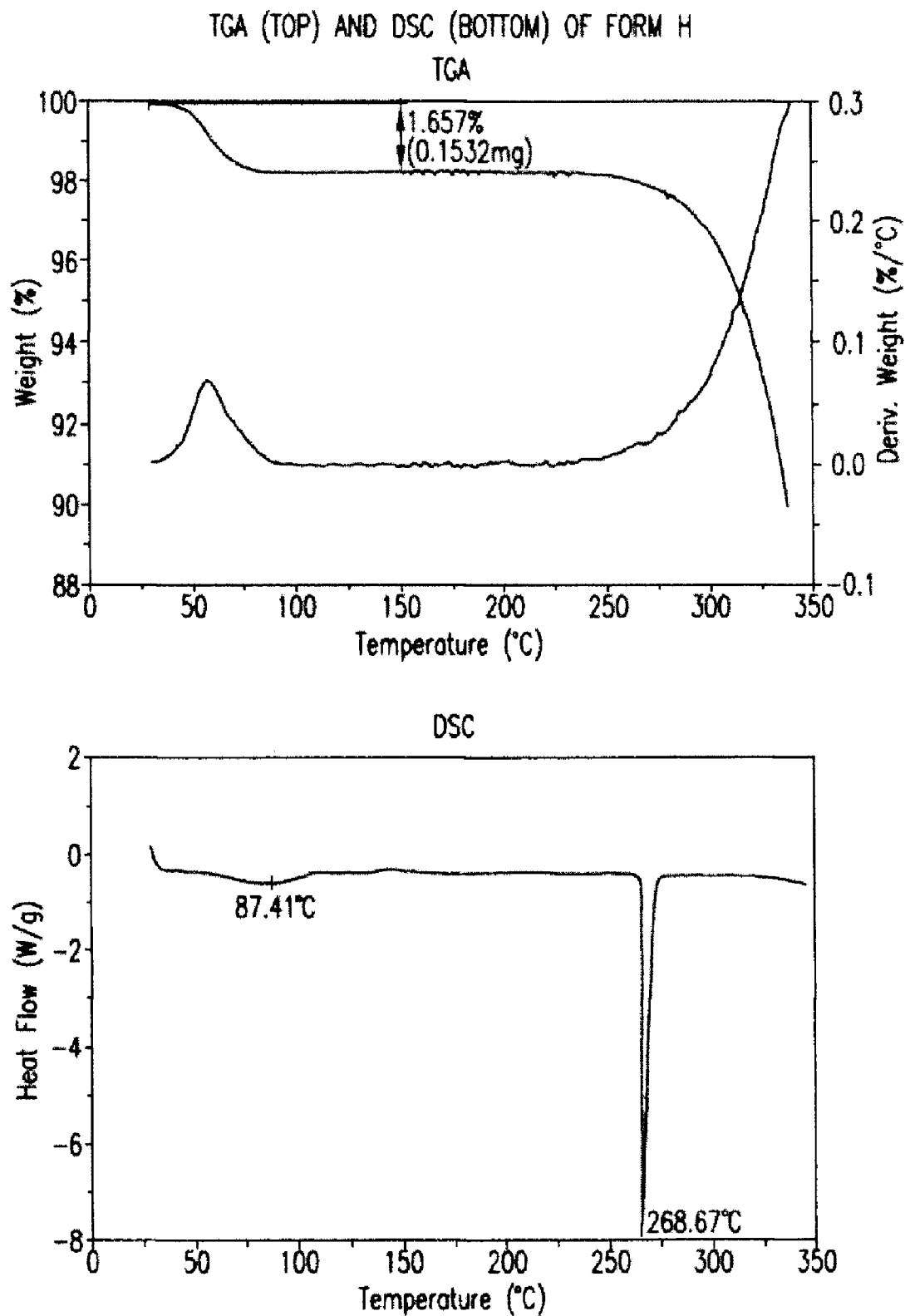


FIG.31

## CERTIFICATE OF CORRECTION (continued)

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## XRPD PATTERN OF POLYMORPH B

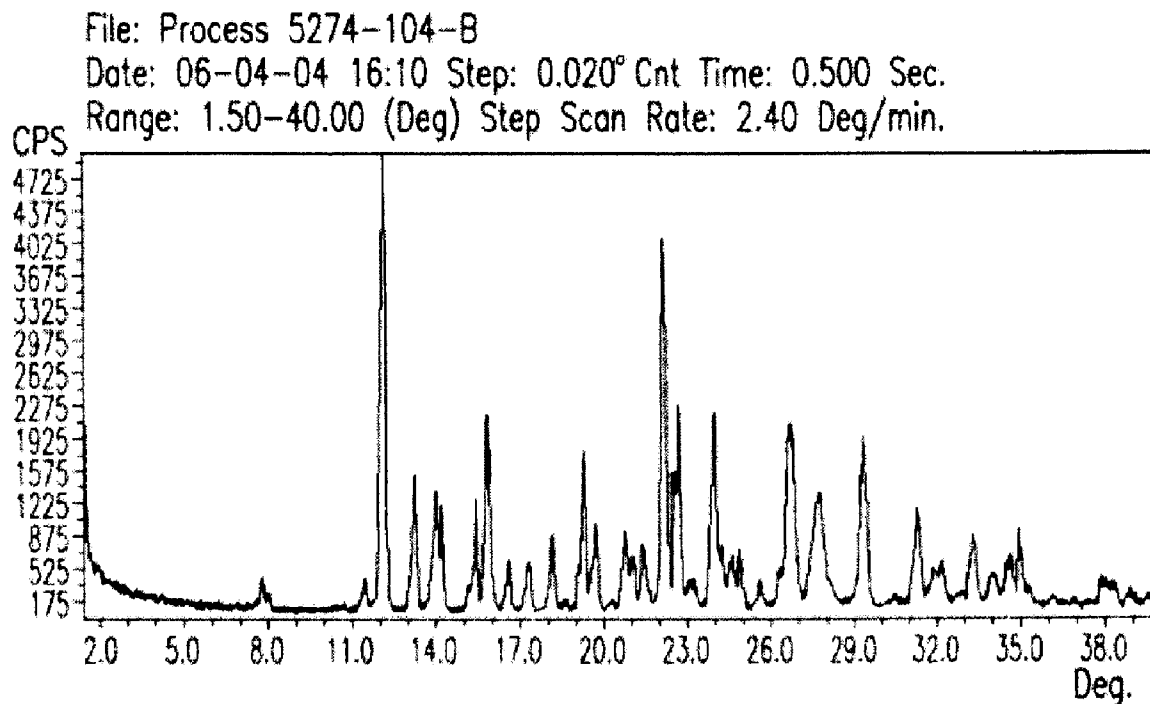


FIG.32

## XRPD PATTERN OF POLYMORPH B

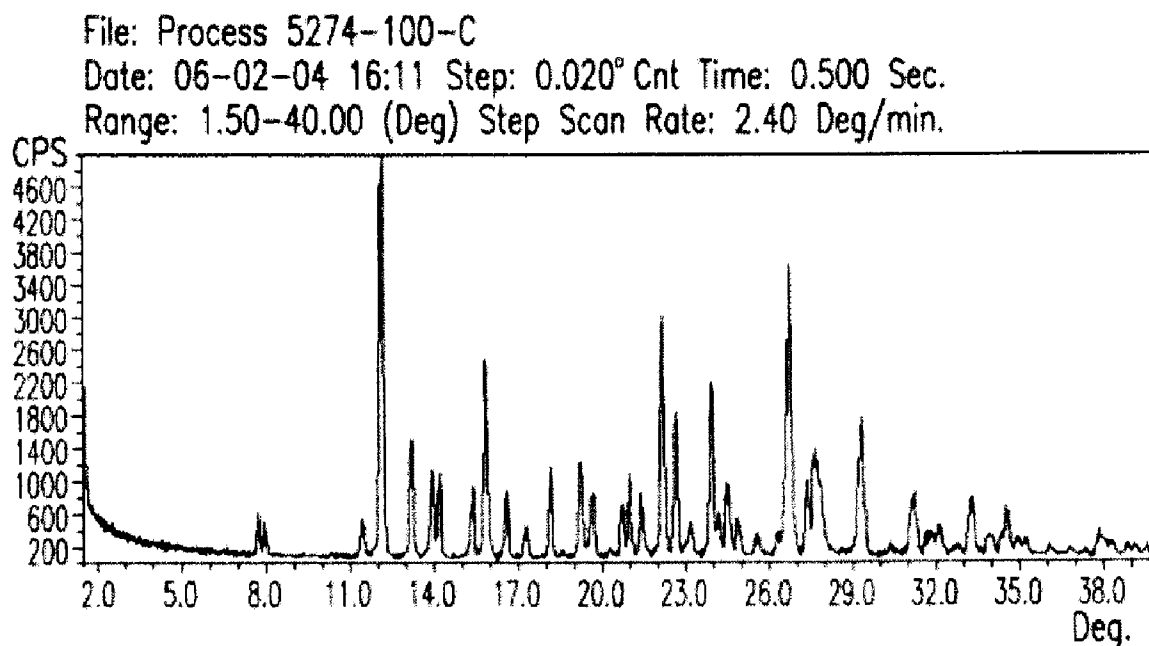


FIG.33

CERTIFICATE OF CORRECTION (continued)

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XRPD PATTERN OF POLYMORPH B

File: Process 5222-157-C

Date: 06/04/04 15:07 Step: 0.020° Cnt Time: 0.500 Sec.

Range: 1.50-40.00 (Deg) Step Scan Rate: 2.40 Deg/min.

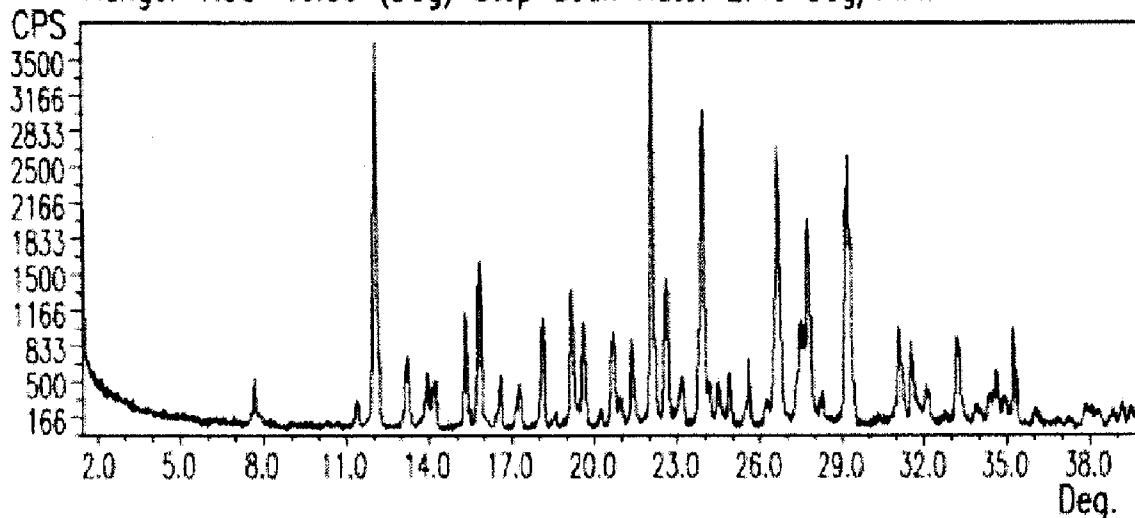


FIG.34

XRPD PATTERN OF POLYMORPH E

File: Process 5222-152-B Form E

Date: 05/21/04 10:46 Step: 0.020° Cnt Time: 0.500 Sec.

Range: 1.50-40.00 (Deg) Step Scan Rate: 2.40 Deg/min.

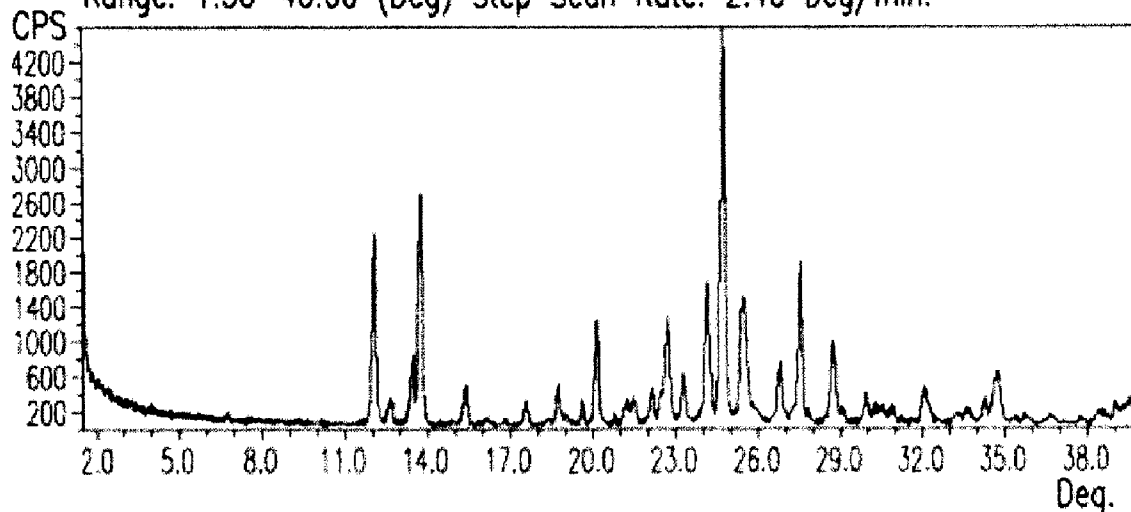


FIG.35

## CERTIFICATE OF CORRECTION (continued)

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TGA CURVE OF POLYMORPH B  
Sample: 5274-104-B  
Size 7.8320mg  
Method: Ramp  
TGA  
Run Date: 09-Jun-04 17:04  
Instrument: TGA 0.500 V5.3 Build 171

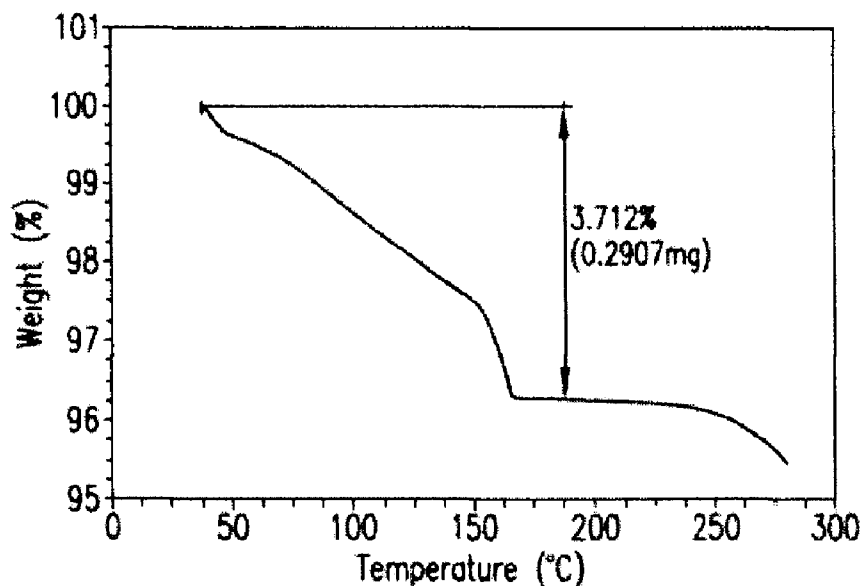


FIG.37

TGA CURVE OF POLYMORPH B  
Sample: 5274-100-C  
Size 10.9430mg  
Method: Ramp  
Comment: Evotec Batch 1675C H2O  
TGA  
Run Date: 03-Jun-04 11:20  
Instrument: TGA 0.500 V5.3 Build 171

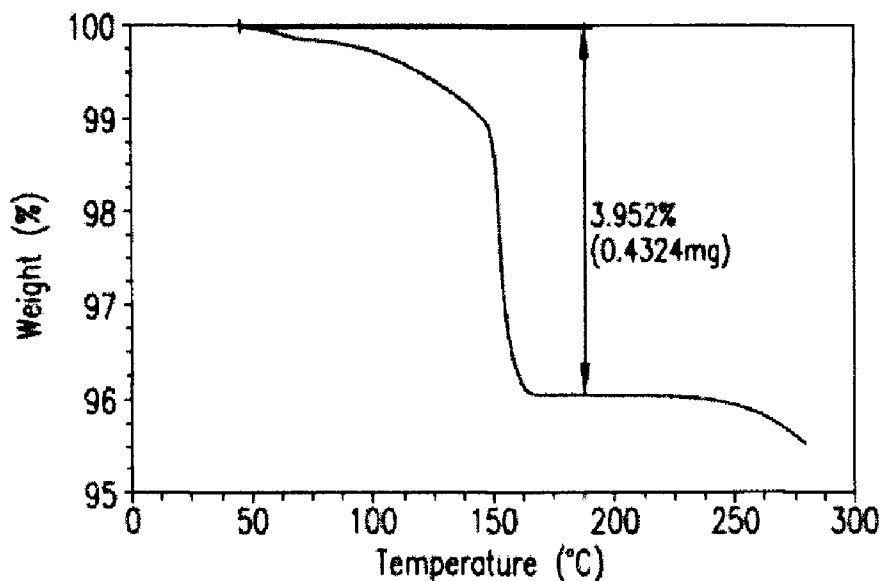


FIG.38

## CERTIFICATE OF CORRECTION (continued)

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TGA CURVE OF POLYMORPH B  
TGA  
Sample: 5222-157-C  
Size: 24.4610mg  
Method: Ramp  
Comment: Evotec Batch 17  
Run Date: 07-Jun-04 09:46  
Instrument: TGA 0.500 V5.3 Build 171

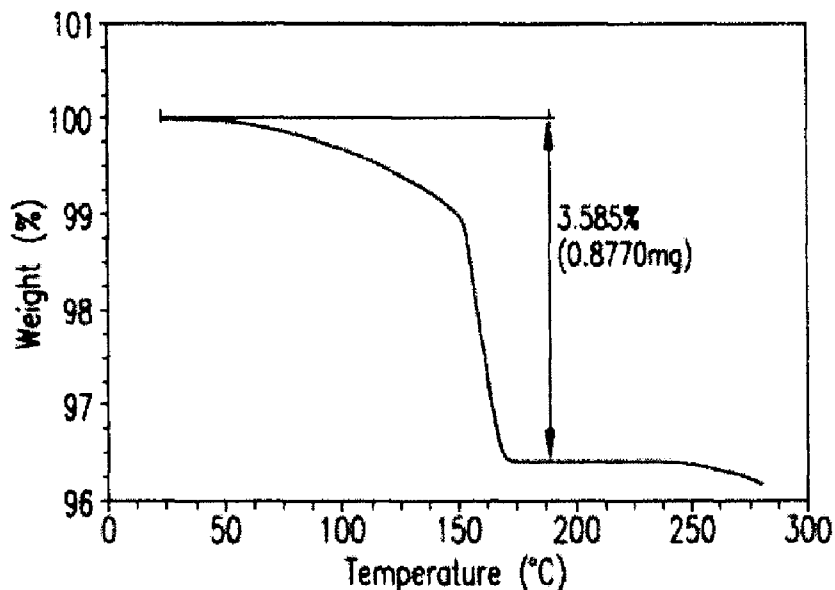


FIG.39

TGA CURVE OF POLYMORPH E  
TGA  
Sample: 5222-152-B  
Size: 11.3850mg  
Method: Ramp  
Comment: Form E  
Run Date: 21-May-04 09:34  
Instrument: TGA 0.500 V5.3 Build 171

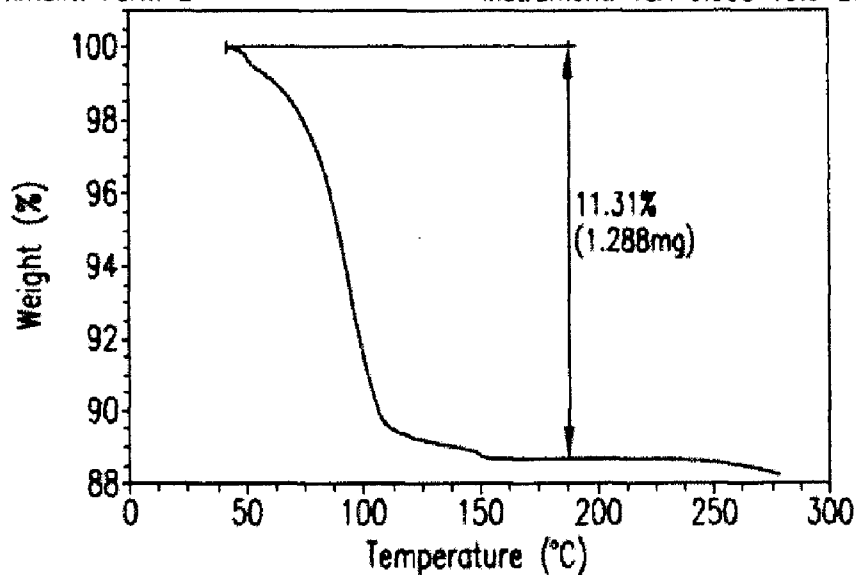


FIG.40

## CERTIFICATE OF CORRECTION (continued)

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DSC OF POLYMORPH B  
DSC  
Sample: 5274-104-B  
Size: 0.0000mg  
Method: Cell constant calibration  
Comment: OAI batch 15 22b  
Run Date: 07-Jun-04 11:30  
Instrument: DSC Q 1000 V7.3 Build 249

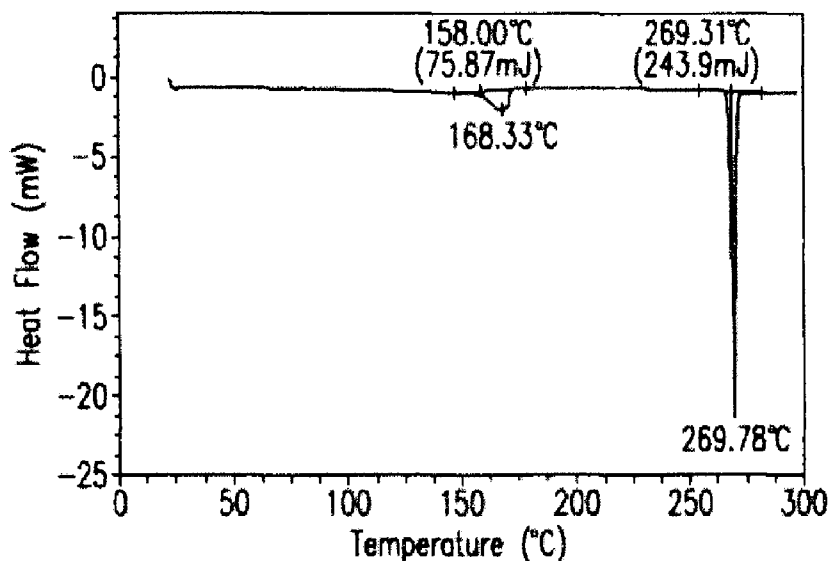


FIG.42

DSC OF POLYMORPH B  
DSC  
Sample: 5274-100-C  
Size: 0.0000mg  
Method: Cell constant calibration  
Comment: Evotec Batch 1675C 10vol H2O 24h 3h dry  
Run Date: 02-Jun-04 17:01  
Instrument: DSC Q 1000 V7.3 Build 249

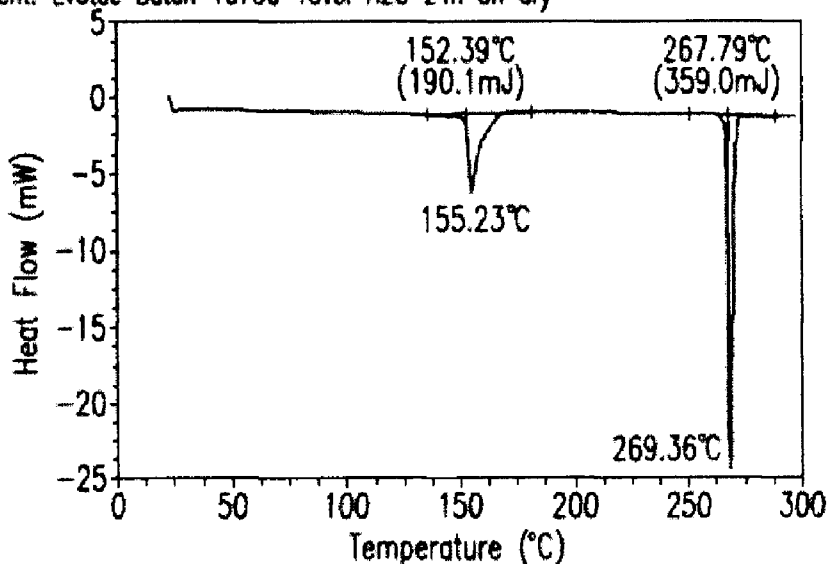


FIG.43

## CERTIFICATE OF CORRECTION (continued)

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DSC OF POLYMORPH B  
DSC

Sample: 5222-157-C  
Size: 0.0000mg  
Method: Cell constant calibration  
Comment: OAI batch 17

Run Date: 07-Jun-04 09:45  
Instrument: DSC Q 1000 V7.3 Build 249

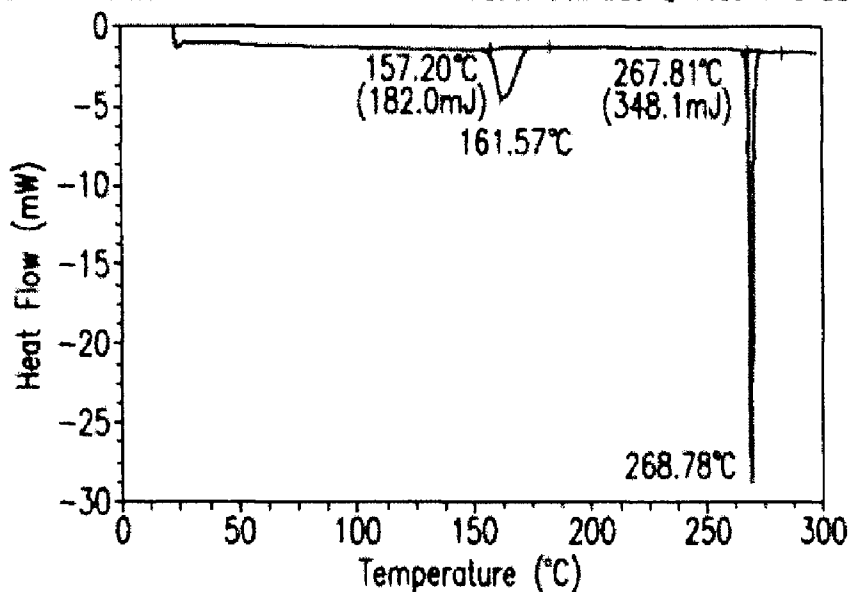


FIG.44

DSC OF POLYMORPH E  
DSC

Sample: 5222-152-B  
Size: 3.4500mg  
Method: Cell constant calibration  
Comment: Evotec batch 15 Form E

Run Date: 21-May-04 09:32  
Instrument: DSC Q 1000 V7.3 Build 249

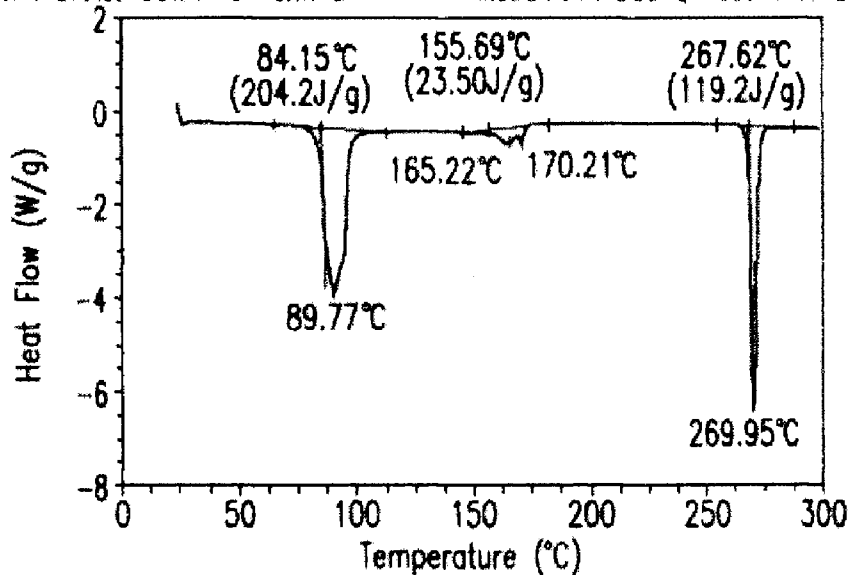


FIG.45

## CERTIFICATE OF CORRECTION (continued)

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DSC OF POLYMORPH MIXTURE  
DSC

Sample: 5222-161-A  
Size: 0.0000mg  
Method: Cell constant calibration  
Comment: OAI batch 15

Run Date: 11-Jun-04 12:44  
Instrument: DSC Q 1000 V7.3 Build 249

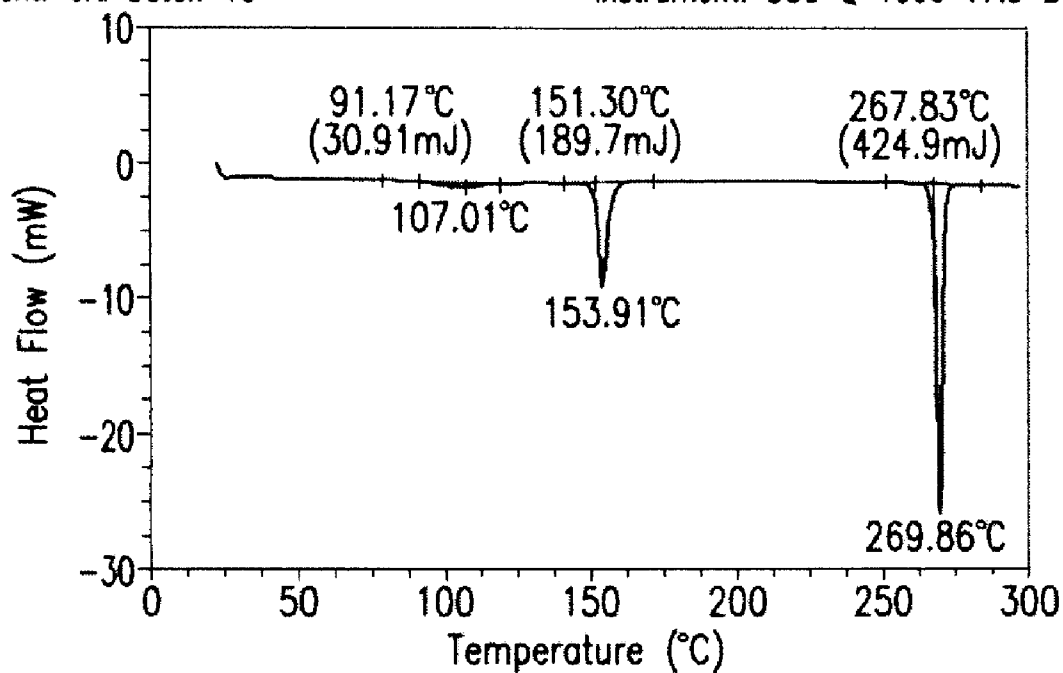


FIG.46



# **EXHIBIT D**



US007968569B2

(12) **United States Patent**  
**Zeldis**

(10) **Patent No.:** **US 7,968,569 B2**  
(45) **Date of Patent:** **Jun. 28, 2011**

(54) **METHODS FOR TREATMENT OF MULTIPLE MYELOMA USING 3-(4-AMINO-1-OXO-1,3-DIHYDRO-ISOINDOL-2-YL)-PIPERIDINE-2,6-DIONE**

(75) Inventor: **Jerome B. Zeldis**, Princeton, NJ (US)

(73) Assignee: **Celgene Corporation**, Summit, NJ (US)

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 145 days.

(21) Appl. No.: **10/438,213**

(22) Filed: **May 15, 2003**

(65) **Prior Publication Data**

US 2004/0029832 A1 Feb. 12, 2004

**Related U.S. Application Data**

(60) Provisional application No. 60/380,842, filed on May 17, 2002, provisional application No. 60/424,600, filed on Nov. 6, 2002.

(51) **Int. Cl.**  
**A61K 31/445** (2006.01)

(52) **U.S. Cl.** ..... **514/323**

(58) **Field of Classification Search** ..... 514/321,  
514/323

See application file for complete search history.

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*Primary Examiner* — Frederick Krass

*Assistant Examiner* — Chris E Simmons

(74) *Attorney, Agent, or Firm* — Jones Day

(57) **ABSTRACT**

Methods of treating, preventing and/or managing cancer as well as and diseases and disorders associated with, or characterized by, undesired angiogenesis are disclosed. Specific methods encompass the administration of an immunomodulatory compound alone or in combination with a second active ingredient. The invention further relates to methods of reducing or avoiding adverse side effects associated with chemotherapy, radiation therapy, hormonal therapy, biological therapy or immunotherapy which comprise the administration of an immunomodulatory compound. Pharmaceutical compositions, single unit dosage forms, and kits suitable for use in methods of the invention are also disclosed.

**15 Claims, 1 Drawing Sheet**

## US 7,968,569 B2

Page 2

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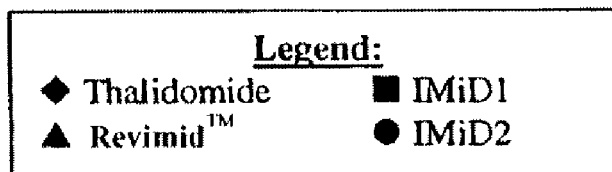
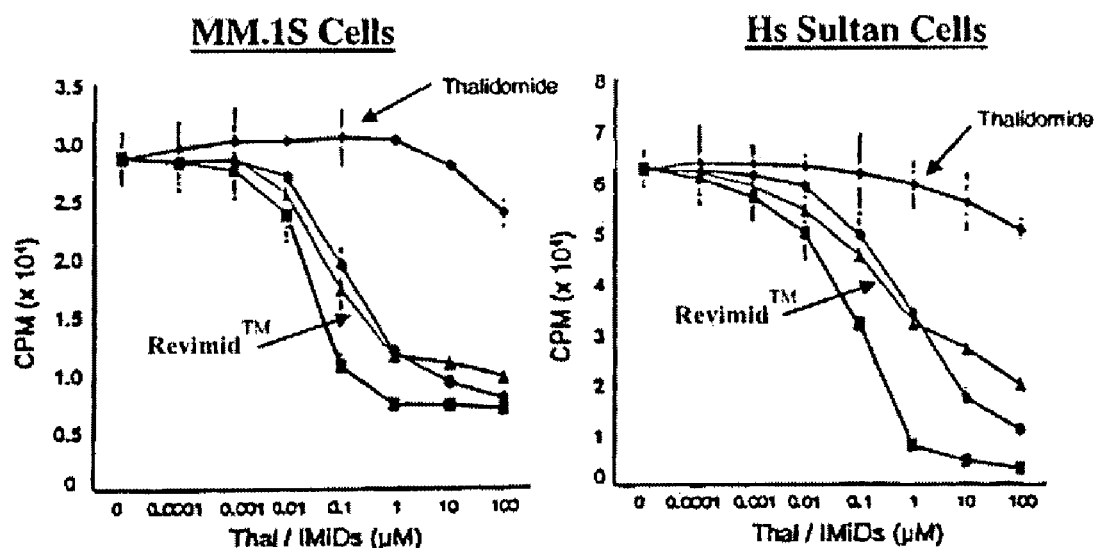
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**Figure 1**  
**Effects of Revimid<sup>TM</sup> and Thalidomide on MM Cell Proliferation**





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# **METHODS FOR TREATMENT OF MULTIPLE MYELOMA USING 3-(4-AMINO-1-OXO-1,3-DIHYDRO-ISOINDOL-2-YL)-PIPERIDINE-2,6-DIONE**

This application claims the benefit of U.S. provisional application No. 60/380,842, filed May 17, 2002, and No. 60/424,600, filed Nov. 6, 2002, the entireties of which are incorporated herein by reference.

## **1. FIELD OF THE INVENTION**

This invention relates to methods of treating, preventing and/or managing specific cancers, and other diseases including, but not limited to, those associated with, or characterized by, undesired angiogenesis, by the administration of one or more immunomodulatory compounds alone or in combination with other therapeutics. In particular, the invention encompasses the use of specific combinations, or "cocktails," of drugs and other therapy, e.g., radiation to treat these specific cancers, including those refractory to conventional therapy. The invention also relates to pharmaceutical compositions and dosing regimens.

## **2. BACKGROUND OF THE INVENTION**

### **2.1 Pathobiology of Cancer and Other Diseases**

Cancer is characterized primarily by an increase in the number of abnormal cells derived from a given normal tissue, invasion of adjacent tissues by these abnormal cells, or lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites (metastasis). Clinical data and molecular biologic studies indicate that cancer is a multistep process that begins with minor preneoplastic changes, which may under certain conditions progress to neoplasia. The neoplastic lesion may evolve clonally and develop an increasing capacity for invasion, growth, metastasis, and heterogeneity, especially under conditions in which the neoplastic cells escape the host's immune surveillance. Roitt, I., Brostoff, J and Kale, D., *Immunology*, 17.1-17.12 (3rd ed., Mosby, St. Louis, Mo., 1993).

There is an enormous variety of cancers which are described in detail in the medical literature. Examples includes cancer of the lung, colon, rectum, prostate, breast, brain, and intestine. The incidence of cancer continues to climb as the general population ages, as new cancers develop, and as susceptible populations (e.g., people infected with AIDS or excessively exposed to sunlight) grow. A tremendous demand therefore exists for new methods and compositions that can be used to treat patients with cancer.

Many types of cancers are associated with new blood vessel formation, a process known as angiogenesis. Several of the mechanisms involved in tumor-induced angiogenesis have been elucidated. The most direct of these mechanisms is the secretion by the tumor cells of cytokines with angiogenic properties. Examples of these cytokines include acidic and basic fibroblastic growth factor (a,b-FGF), angiogenin, vascular endothelial growth factor (VEGF), and TNF- $\alpha$ . Alternatively, tumor cells can release angiogenic peptides through the production of proteases and the subsequent breakdown of the extracellular matrix where some cytokines are stored (e.g., b-FGF). Angiogenesis can also be induced indirectly through the recruitment of inflammatory cells (particularly macrophages) and their subsequent release of angiogenic cytokines (e.g., TNF- $\alpha$ , bFGF).

A variety of other diseases and disorders are also associated with, or characterized by, undesired angiogenesis. For

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example, enhanced or unregulated angiogenesis has been implicated in a number of diseases and medical conditions including, but not limited to, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, rubeosis (neovascularization of the angle), viral diseases, genetic diseases, inflammatory diseases, allergic diseases, and autoimmune diseases. Examples of such diseases and conditions include, but are not limited to: diabetic retinopathy; retinopathy of prematurity; corneal graft rejection; neovascular glaucoma; retrolental fibroplasia; and proliferative vitreoretinopathy.

Accordingly, compounds that can control angiogenesis or inhibit the production of certain cytokines, including TNF- $\alpha$ , may be useful in the treatment and prevention of various diseases and conditions.

### **2.2 Methods of Treating Cancer**

Current cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient (see, for example, Stockdale, 1998, *Medicine*, vol. 3, Rubenstein and Federman, eds., Chapter 12, Section IV). Recently, cancer therapy could also involve biological therapy or immunotherapy. All of these approaches pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of a patient or may be unacceptable to the patient. Additionally, surgery may not completely remove neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue. Radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent. Although hormonal therapy can be effective, it is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of cancer cells. Biological therapies and immunotherapies are limited in number and may produce side effects such as rashes or swellings, flu-like symptoms, including fever, chills and fatigue, digestive tract problems or allergic reactions.

With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A majority of cancer chemotherapeutics act by inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division. Gilman et al., *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, Tenth Ed. (McGraw Hill, New York).

Despite availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks. Stockdale, *Medicine*, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. 10, 1998. Almost all chemotherapeutic agents are toxic, and chemotherapy causes significant, and often dangerous side effects including severe nausea, bone marrow depression, and immunosuppression. Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even if those agents act by different mechanism from those of the drugs used in the specific treatment. This phenomenon is referred to as pleiotropic drug or multidrug resistance. Because of the drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

Other diseases or conditions associated with, or characterized by, undesired angiogenesis are also difficult to treat. However, some compounds such as protamine, heparin and steroids have been proposed to be useful in the treatment of certain specific diseases. Taylor et al., *Nature* 297:307 (1982);

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Folkman et al., *Science* 221:719 (1983); and U.S. Pat. Nos. 5,001,116 and 4,994,443. Thalidomide and certain derivatives of it have also been proposed for the treatment of such diseases and conditions. U.S. Pat. Nos. 5,593,990, 5,629,327, 5,712,291, 6,071,948 and 6,114,355 to D'Amato.

Still, there is a significant need for safe and effective methods of treating, preventing and managing cancer and other diseases and conditions, particularly for diseases that are refractory to standard treatments, such as surgery, radiation therapy, chemotherapy and hormonal therapy, while reducing or avoiding the toxicities and/or side effects associated with the conventional therapies.

### 2.3 IMIDS™

A number of studies have been conducted with the aim of providing compounds that can safely and effectively be used to treat diseases associated with abnormal production of TNF- $\alpha$ . See, e.g., Marriott, J. B., et al., *Expert Opin. Biol. Ther.* 1(4):1-8 (2001); G. W. Muller, et al., *Journal of Medicinal Chemistry* 39(17): 3238-3240 (1996); and G. W. Muller, et al, *Bioorganic & Medicinal Chemistry Letters* 8: 2669-2674 (1998). Some studies have focused on a group of compounds selected for their capacity to potentially inhibit TNF- $\alpha$  production by LPS stimulated PBMC. L. G. Corral, et al., *Ann. Rheum. Dis.* 58:(Suppl 1) 1107-1113 (1999). These compounds, which are referred to as IMiDS™ (Celgene Corporation) or Immunomodulatory Drugs, show not only potent inhibition of TNF- $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  and IL12 production. LPS induced IL6 is also inhibited by immunomodulatory compounds, albeit partially. These compounds are potent stimulators of LPS induced IL10. Id. Particular examples of IMiDS™s include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles described in U.S. Pat. Nos. 6,281,230 and 6,316,471, both to G. W. Muller, et al.

### 3. SUMMARY OF THE INVENTION

This invention encompasses methods of treating and preventing certain types of cancer, including primary and metastatic cancer, as well as cancers that are refractory or resistant to conventional chemotherapy. The methods comprise administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. The invention also encompasses methods of managing certain cancers (e.g., preventing or prolonging their recurrence, or lengthening the time of remission) which comprise administering to a patient in need of such management a prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage cancer. Examples of such conventional therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention also encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are associated with, or characterized by, undesired angiogenesis, which comprise administering to a patient in need of such treatment, management or prevention a therapeutically or prophylactically effective amount of an immunomodula-

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tory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In other methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage diseases or disorders associated with, or characterized by, undesired angiogenesis. Examples of such conventional therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention encompasses pharmaceutical compositions, single unit dosage forms, dosing regimens and kits which comprise an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second, or additional, active agent. Second active agents include specific combinations, or "cocktails," of drugs.

### 4. BRIEF DESCRIPTION OF FIGURE

FIG. 1 shows a comparison of the effects of 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide in inhibiting the proliferation of multiple myeloma (MM) cell lines in an in vitro study. The uptake of [<sup>3</sup>H]-thymidine by different MM cell lines (MM. 1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of the cell proliferation.

### 5. DETAILED DESCRIPTION OF THE INVENTION

A first embodiment of the invention encompasses methods of treating, managing, or preventing cancer which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with another drug ("second active agent") or method of treating, managing, or preventing cancer. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage cancer.

Another embodiment of the invention encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are characterized by undesired angiogenesis. These methods comprise the administration of a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

Examples of diseases and disorders associated with, or characterized by, undesired angiogenesis include, but are not limited to, inflammatory diseases, autoimmune diseases, viral diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, and rubeosis (neovascularization of the angle).

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combina-

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tion with a second active agent or method of treating, managing, or preventing the disease or condition. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage disease and conditions associated with, or characterized by, undesired angiogenesis.

The invention also encompasses pharmaceutical compositions (e.g., single unit dosage forms) that can be used in methods disclosed herein. Particular pharmaceutical compositions comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second active agent.

#### 5.1 Immunomodulatory Compounds

Compounds used in the invention include immunomodulatory compounds that are racemic, stereomerically enriched or stereomerically pure, and pharmaceutically acceptable salts, solvates, hydrates, stereoisomers, clathrates, and prodrugs thereof. Preferred compounds used in the invention are small organic molecules having a molecular weight less than about 1,000 g/mol, and are not proteins, peptides, oligonucleotides, oligosaccharides or other macromolecules.

As used herein and unless otherwise indicated, the terms "immunomodulatory compounds" and "IMiDs™" (Celgene Corporation) encompasses small organic molecules that markedly inhibit TNF- $\alpha$ , LPS induced monocyte IL1 $\beta$  and IL12, and partially inhibit IL6 production. Specific immunomodulatory compounds are discussed below.

TNF- $\alpha$  is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation. TNF- $\alpha$  is responsible for a diverse range of signaling events within cells. TNF- $\alpha$  may play a pathological role in cancer. Without being limited by theory, one of the biological effects exerted by the immunomodulatory compounds of the invention is the reduction of synthesis of TNF- $\alpha$ . Immunomodulatory compounds of the invention enhance the degradation of TNF- $\alpha$ mRNA.

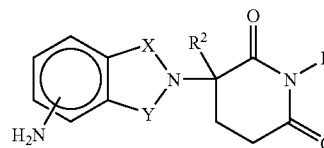
Further, without being limited by theory, immunomodulatory compounds used in the invention may also be potent co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. Immunomodulatory compounds of the invention may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset. In addition, the compounds preferably have anti-inflammatory properties, and efficiently co-stimulate T cells.

Specific examples of immunomodulatory compounds of the invention, include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl) isoindolines such as those described in U.S. Pat. No. 5,874,448; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolines described in U.S. Pat. No. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines (e.g., 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517; and a class of non-peptide cyclic amides disclosed in U.S. Pat. Nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S.

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Pat. Nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles such as those described in U.S. Pat. Nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those described in U.S. patent application Ser. No. 09/972,487 filed on Oct. 5, 2001, U.S. patent application Ser. No. 10/032,286 filed on Dec. 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents and patent applications identified herein are incorporated herein by reference. Immunomodulatory compounds of the invention do not include thalidomide.

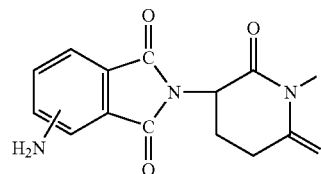
Other specific immunomodulatory compounds of the invention include, but are not limited to, 1-oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein by reference. These compounds have the structure I:



in which one of X and Y is C=O, the other of X and Y is C=O or CH<sub>2</sub>, and R<sup>2</sup> is hydrogen or lower alkyl, in particular methyl. Specific immunomodulatory compounds include, but are not limited to:

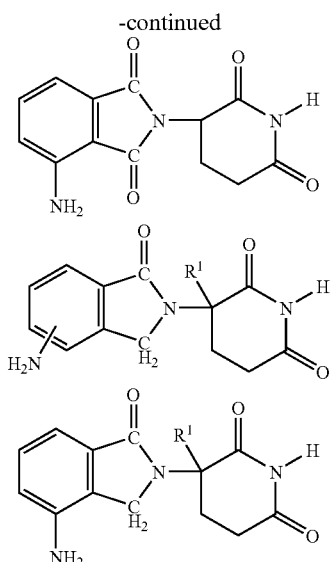
1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;  
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
and  
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

Other specific immunomodulatory compounds of the invention belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles, such as those described in U.S. Pat. Nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (International Publication No. WO 98/03502), each of which is incorporated herein by reference. Compounds representative of this class are of the formulas:



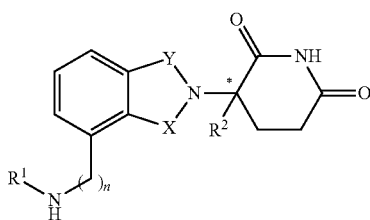
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wherein  $R^1$  is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (e.g. optically pure (R) or (S) enantiomers) of these compounds.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. Nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are incorporated herein by reference. Representative compounds are of formula II:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is  $C=O$  and the other is  $CH_2$  or  $C\equiv O$ ;

$R^1$  is H,  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $C(O)R^3$ ,  $C(S)R^3$ ,  $C(O)OR^4$ ,  $(C_1-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $C(O)NHR^3$ ,  $C(S)NHR^3$ ,  $C(O)NR^3R^{3'}$ ,  $C(S)NR^3R^{3'}$  or  $(C_1-C_8)$ alkyl- $O(CO)R^5$ ;

$R^2$  is H, F, benzyl,  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl, or  $(C_2-C_8)$ alkynyl;

$R^3$  and  $R^{3'}$  are independently  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_0-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $(C_1-C_8)$ alkyl- $O(CO)R^5$ , or  $C(O)OR^5$ ;

$R^4$  is  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl,  $(C_1-C_4)$ alkyl- $OR^5$ , benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl, or  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl;

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$R^5$  is  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl, or  $(C_2-C_5)$ heteroaryl;

each occurrence of  $R^6$  is independently H,  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_2-C_5)$ heteroaryl, or  $(C_0-C_8)$ alkyl- $C(O)OR^5$  or the  $R^6$  groups can join to form a heterocycloalkyl group;

n is 0 or 1; and

\* represents a chiral-carbon center.

In specific compounds of formula II, when n is 0 then  $R^1$  is  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $C(O)R^3$ ,  $C(O)OR^4$ ,  $(C_1-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $C(S)NHR^3$ , or  $(C_1-C_8)$ alkyl- $O(CO)R^5$ ;

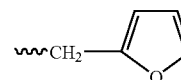
$R^2$  is H or  $(C_1-C_8)$ alkyl; and

$R^3$  is  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_5-C_8)$ alkyl- $N(R^6)_2$ ;  $(C_0-C_8)$ alkyl- $NH-C(O)OR^5$ ;  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $(C_1-C_8)$ alkyl- $O(CO)R^5$ , or  $C(O)OR^5$ ; and the other variables have the same definitions.

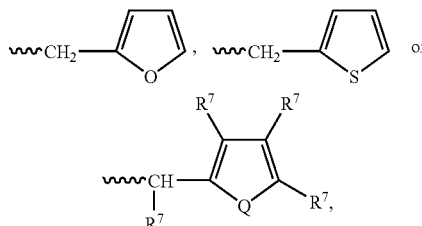
In other specific compounds of formula II,  $R^2$  is H or  $(C_1-C_4)$ alkyl.

In other specific compounds of formula II,  $R^1$  is  $(C_1-C_8)$ alkyl or benzyl.

In other specific compounds of formula II,  $R^1$  is H,  $(C_1-C_8)$ alkyl, benzyl,  $CH_2OCH_3$ ,  $CH_2CH_2OCH_3$ , or



In another embodiment of the compounds of formula II,  $R^1$  is



wherein Q is O or S, and each occurrence of  $R^7$  is independently H,  $(C_1-C_8)$ alkyl, benzyl,  $CH_2OCH_3$ , or  $CH_2CH_2OCH_3$ .

In other specific compounds of formula II,  $R^1$  is  $C(O)R^3$ .

In other specific compounds of formula II,  $R^3$  is  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_1-C_5)$ alkyl, aryl, or  $(C_0-C_4)$ alkyl- $OR^5$ .

In other specific compounds of formula II, heteroaryl is pyridyl, furyl, or thienyl.

In other specific compounds of formula II,  $R^1$  is  $C(O)OR^4$ .

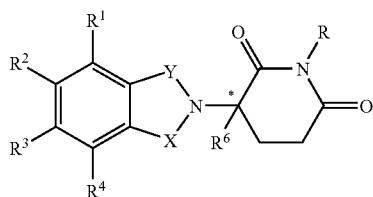
In other specific compounds of formula II, the H of  $C(O)NHC(O)$  can be replaced with  $(C_1-C_4)$ alkyl, aryl, or benzyl.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. No. 09/781,179, International Publication No. WO 98/54170, and U.S. Pat. No. 6,395,754, each of which are incorporated herein by reference. Representative compounds are of formula III:



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and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

R is H or CH<sub>2</sub>OCOR';

(i) each of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup>, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> is nitro or —NHR<sup>5</sup> and the remaining of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> are hydrogen;

R<sup>5</sup> is hydrogen or alkyl of 1 to 8 carbons

R<sup>6</sup> hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro;

R' is R<sup>7</sup>—CHR<sup>10</sup>—N(R<sup>8</sup>R<sup>9</sup>);

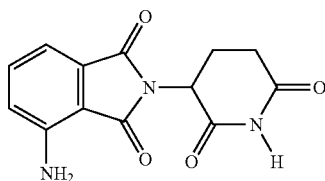
R<sup>7</sup> is m-phenylene or p-phenylene or —(C<sub>n</sub>H<sub>2n</sub>)— in which n has a value of 0 to 4;

each of R<sup>8</sup> and R<sup>9</sup> taken independently of the other is hydrogen or alkyl of 1 to 8 carbon atoms, or R<sup>8</sup> and R<sup>9</sup> taken together are tetramethylene, pentamethylene, hexamethylene, or —CH<sub>2</sub>CH<sub>2</sub>[X]<sub>1</sub>CH<sub>2</sub>CH<sub>2</sub>— in which [X]<sub>1</sub> is —O—, —S—, or —NH—;

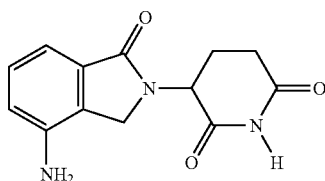
R<sup>10</sup> is hydrogen, alkyl of 1 to 8 carbon atoms, or phenyl; and

\* represents a chiral-carbon center.

The most preferred immunomodulatory compounds of the invention are 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. The compounds can be obtained via standard, synthetic methods (see e.g., U.S. Pat. No. 5,635, 517, incorporated herein by reference). The compounds are available from Celgene Corporation, Warren, N.J. 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (ACTIMID™) has the following chemical structure:



The compound 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (REVIMID™) has the following chemical structure:



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Compounds of the invention can either be commercially purchased or prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry techniques.

As used herein and unless otherwise indicated, the term “pharmaceutically acceptable salt” encompasses non-toxic acid and base addition salts of the compound to which the term refers. Acceptable non-toxic acid addition salts include those derived from organic and inorganic acids or bases known in the art, which include, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embolic acid, enanthic acid, and the like.

Compounds that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds are those that form non-toxic base addition salts, i.e., salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), lysine, and procaine.

As used herein and unless otherwise indicated, the term “prodrug” means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of immunomodulatory compounds of the invention that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of immunomodulatory compounds of the invention that comprise —NO, —NO<sub>2</sub>, —ONO, or —ONO<sub>2</sub> moieties. Prodrugs can typically be prepared using well-known methods, such as those described in 1 *Burger's Medicinal Chemistry and Drug Discovery*, 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995), and *Design of Prodrugs* (H. Bundgaard ed., Elsevier, N.Y. 1985).

As used herein and unless otherwise indicated, the terms “biohydrolyzable amide,” “biohydrolyzable ester,” “biohydrolyzable carbamate,” “biohydrolyzable carbonate,” “biohydrolyzable ureide,” “biohydrolyzable phosphate” mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxymethyl, acetoxylethyl, aminocarbonyloxymethyl, pivaloyloxymethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyl-oxymethyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides

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include, but are not limited to, lower alkyl amides,  $\alpha$ -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, amino acids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

Various immunomodulatory compounds of the invention contain one or more chiral centers, and can exist as racemic mixtures of enantiomers or mixtures of diastereomers. This invention encompasses the use of stereomerically pure forms of such compounds, as well as the use of mixtures of those forms. For example, mixtures comprising equal or unequal amounts of the enantiomers of a particular immunomodulatory compounds of the invention may be used in methods and compositions of the invention. These isomers may be asymmetrically synthesized or resolved using standard techniques such as chiral columns or chiral resolving agents. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, N.Y., 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. As used herein and unless otherwise indicated, the term "stereomerically enriched" means a composition that comprises greater than about 60% by weight of one stereoisomer of a compound, preferably greater than about 70% by weight, more preferably greater than about 80% by weight of one stereoisomer of a compound. As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center. Similarly, the term "stereomerically enriched" means a stereomerically enriched composition of a compound having one chiral center.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

#### 5.2 Second Active Agents

Immunomodulatory compounds can be combined with other pharmacologically active compounds ("second active agents") in methods and compositions of the invention. It is

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believed that certain combinations work synergistically in the treatment of particular types of cancer and certain diseases and conditions associated with, or characterized by, undesired angiogenesis. Immunomodulatory compounds can also work to alleviate adverse effects associated with certain second active agents, and some second active agents can be used to alleviate adverse effects associated with immunomodulatory compounds.

One or more second active ingredients or agents can be used in the methods and compositions of the invention together with an immunomodulatory compound. Second active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules).

Examples of large molecule active agents include, but are not limited to, hematopoietic growth factors, cytokines, and monoclonal and polyclonal antibodies. Typical large molecule active agents are biological molecules, such as naturally occurring or artificially made proteins. Proteins that are particularly useful in this invention include proteins that stimulate the survival and/or proliferation of hematopoietic precursor cells and immunologically active poietic cells in vitro or in vivo. Others stimulate the division and differentiation of committed erythroid progenitors in cells in vitro or in vivo. Particular proteins include, but are not limited to: interleukins, such as IL-2 (including recombinant IL-2 ("rIL2") and canarypox IL-2), IL-10, IL-12, and IL-18; interferons, such as interferon alfa-2a, interferon alfa-2b, interferon alfa-n1, interferon alfa-n3, interferon beta-I a, and interferon gamma-I b; GM-CSF and GM-CSF; and EPO.

Particular proteins that can be used in the methods and compositions of the invention include, but are not limited to: filgrastim, which is sold in the United States under the trade name Neupogen® (Amgen, Thousand Oaks, Calif.); sargramostim, which is sold in the United States under the trade name Leukine® (Immunex, Seattle, Wash.); and recombinant EPO, which is sold in the United States under the trade name Epogen® (Amgen, Thousand Oaks, Calif.).

Recombinant and mutated forms of GM-CSF can be prepared as described in U.S. Pat. Nos. 5,391,485; 5,393,870; and 5,229,496; all of which are incorporated herein by reference. Recombinant and mutated forms of G-CSF can be prepared as described in U.S. Pat. Nos. 4,810,643; 4,999,291; 5,528,823; and 5,580,755; all of which are incorporated herein by reference.

This invention encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term "mutants" are proteins that lack carbohydrate moieties normally present in their naturally occurring forms (e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001).

Antibodies that can be used in combination with compounds of the invention include monoclonal and polyclonal antibodies. Examples of antibodies include, but are not limited to, trastuzumab (Herceptin®), rituximab (Rituxan®), bevacizumab (Avastin™), pertuzumab (Omnitarg™), tositu-

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momab (Bexxar®), edrecolomab (Panorex®), and G250. Compounds of the invention can also be combined with, or used in combination with, anti-TNF- $\alpha$  antibodies.

Large molecule active agents may be administered in the form of anti-cancer vaccines. For example, vaccines that secrete, or cause the secretion of, cytokines such as IL-2, G-CSF, and GM-CSF can be used in the methods, pharmaceutical compositions, and kits of the invention. See, e.g., Emens, L. A., et al., *Curr. Opinion Mol. Ther.* 3(1):77-84 (2001).

In one embodiment of the invention, the large molecule active agent reduces, eliminates, or prevents an adverse effect associated with the administration of an immunomodulatory compound. Depending on the particular immunomodulatory compound and the disease or disorder being treated, adverse effects can include, but are not limited to, drowsiness and somnolence, dizziness and orthostatic hypotension, neutropenia, infections that result from neutropenia, increased HIV-viral load, bradycardia, Stevens-Johnson Syndrome and toxic epidermal necrolysis, and seizures (e.g., grand mal convulsions). A specific adverse effect is neutropenia.

Second active agents that are small molecules can also be used to alleviate adverse effects associated with the administration of an immunomodulatory compound. However, like some large molecules, many are believed to be capable of providing a synergistic effect when administered with (e.g., before, after or simultaneously) an immunomodulatory compound. Examples of small molecule second active agents include, but are not limited to, anti-cancer agents, antibiotics, immunosuppressive agents, and steroids.

Examples of anti-cancer agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; amet-antrone acetate; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; celecoxib (COX-2 inhibitor); chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziqune; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflomithine hydrochloride; elsamitucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; iproplatin; irinotecan; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipo-

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broman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procabazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiro-mustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; taxotere; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; tricinribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulazole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vingly-cinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; and zorubicin hydrochloride.

Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinates; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlins; chloroquinoline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatan; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrididemnin B; deslorelin; dexamethasone; dexifosfamide; dextrazoxane; dexverapamil; diaziqune; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; doxorubicin; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflomithine; elemene; emitofur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium tetraphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofofosine; ilomastat; imatinib (e.g., Gleevec®), imiquimod; immunostimulant peptides;



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insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannosatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mitoguanzone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; Erbitux, human chorionic gonadotrophin; monophosphoryl lipid A+mycobacterium cell wall sk; mopidamol; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; narogrostim; nedaplatin; nemorubicin; neridronic acid; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; oblimersen (Genasense®); O<sup>6</sup>-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytiol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras famesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; translation inhibitors; tretinoin; triacetyluridine; tricirbine;

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trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Specific second active agents include, but are not limited to, oblimersen (Genasense®), remicade, docetaxel, celecoxib, melphalan, dexamethasone (Decadron®), steroids, gemcitabine, cisplatin, temozolomide, etoposide, cyclophosphamide, temodar, carboplatin, procarbazine, gliadel, tamoxifen, topotecan, methotrexate, Arisa®, taxol, taxotere, fluorouracil, leucovorin, irinotecan, xeloda, CPT-11, interferon alpha, pegylated interferon alpha (e.g., PEG INTRON-A), capecitabine, cisplatin, thiopeta, fludarabine, carboplatin, liposomal daunorubicin, cytarabine, doxetaxol, paclitaxel, vinblastine, IL-2, GM-CSF, dacarbazine, vinorelbine, zoledronic acid, palmitronate, biaxin, busulphan, prednisone, bisphosphonate, arsenic trioxide, vincristine, doxorubicin (Doxil®), paclitaxel, ganciclovir, adriamycin, estramustine sodium phosphate (Emcyt®), sulindac, and etoposide.

### 5.3 Methods of Treatments and Prevention

Methods of this invention encompass methods of treating, preventing and/or managing various types of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis. As used herein, unless otherwise specified, the term “treating” refers to the administration of a compound of the invention or other additional active agent after the onset of symptoms of the particular disease or disorder. As used herein, unless otherwise specified, the term “preventing” refers to the administration prior to the onset of symptoms, particularly to patients at risk of cancer, and other diseases and disorders associated with, or characterized by, undesired angiogenesis. The term “prevention” includes the inhibition of a symptom of the particular disease or disorder. Patients with familial history of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis are preferred candidates for preventive regimens. As used herein and unless otherwise indicated, the term “managing” encompasses preventing the recurrence of the particular disease or disorder in a patient who had suffered from it, and/or lengthening the time a patient who had suffered from the disease or disorder remains in remission.

As used herein, the term “cancer” includes, but is not limited to, solid tumors and blood born tumors. The term “cancer” refers to disease of skin tissues, organs, blood, and vessels, including, but not limited to, cancers of the bladder, bone or blood, brain, breast, cervix, chest, colon, endometrium, esophagus, eye, head, kidney, liver, lymph nodes, lung, mouth, neck, ovaries, pancreas, prostate, rectum, stomach, testis, throat, and uterus. Specific cancers include, but are not limited to, advanced malignancy, amyloidosis, neuroblastoma, meningioma, hemangiopericytoma, multiple brain metastase, glioblastoma multiforms, glioblastoma, brain stem glioma, poor prognosis malignant brain tumor, malignant glioma, recurrent malignant glioma, anaplastic astrocytoma, anaplastic oligodendroglioma, neuroendocrine tumor, rectal adenocarcinoma, Dukes C & D colorectal cancer, unresectable colorectal carcinoma, metastatic hepatocellular carcinoma, Kaposi’s sarcoma, karotype acute myeloblastic leukemia, Hodgkin’s lymphoma, non-Hodgkin’s lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma, low grade follicular lymphoma, malignant melanoma, malignant mesothelioma, malignant pleural effusion mesothelioma syndrome, peritoneal carcinoma, papillary serous carcinoma, gynec-

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logic sarcoma, soft tissue sarcoma, scleroderma, cutaneous vasculitis, Langerhans cell histiocytosis, leiomyosarcoma, fibrodysplasia ossificans progressive, hormone refractory prostate cancer, resected high-risk soft tissue sarcoma, unresectable hepatocellular carcinoma, Waldenström's macroglobulinemia, smoldering myeloma, indolent myeloma, fallopian tube cancer, androgen independent prostate cancer, androgen dependent stage IV non-metastatic prostate cancer, hormone-insensitive prostate cancer, chemotherapy-insensitive prostate cancer, papillary thyroid carcinoma, follicular thyroid carcinoma, medullary thyroid carcinoma, and leiomyoma. In a specific embodiment, the cancer is metastatic. In another embodiment, the cancer is refractory or resistance to chemotherapy or radiation; in particular, refractory to thalidomide.

As used herein to refer to diseases and conditions other than cancer, the terms "diseases or disorders associated with, or characterized by, undesired angiogenesis," "diseases or disorders associated with undesired angiogenesis," and "diseases or disorders characterized by undesired angiogenesis" refer to diseases, disorders and conditions that are caused, mediated or attended by undesired, unwanted or uncontrolled angiogenesis, including, but not limited to, inflammatory diseases, autoimmune diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroïdal neovascular diseases, and retina neovascular diseases.

Examples of such diseases or disorders associated with undesired angiogenesis include, but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, proliferative vitreoretinopathy, trachoma, myopia, optic pits, epidemic keratoconjunctivitis, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjögrens, acne rosacea, phlyctenulosis, syphilis, lipid degeneration, bacterial ulcer, fungal ulcer, Herpes simplex infection, Herpes zoster infection, protozoan infection, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Bechets disease, retinitis, choroiditis, presumed ocular histoplasmosis, Bests disease, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, rubeosis, sarcoidosis, sclerosis, soriatis, psoriasis, primary sclerosing cholangitis, proctitis, primary biliary srosis, idiopathic pulmonary fibrosis, and alcoholic hepatitis.

In specific embodiments of the invention, diseases or disorders associated with undesired angiogenesis do not include congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, myocardial infarction, HIV, hepatitis, adult respiratory distress syndrome, bone-resorption disease, chronic obstructive pulmonary diseases, chronic pulmonary inflammatory disease, dermatitis, cystic fibrosis, septic shock, sepsis, endotoxic shock, hemodynamic shock, sepsis syndrome, post ischemic reperfusion injury, meningitis, psoriasis, fibrotic disease, cachexia, graft rejection, rheumatoid spondylitis, osteoporosis, Crohn's disease, ulcerative colitis, inflammatory-bowel disease, multiple sclerosis, systemic lupus erythrematosus, erythema nodosum leprosum in leprosy, radiation damage, asthma, hyperoxic alveolar injury, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

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This invention encompasses methods of treating patients who have been previously treated for cancer or diseases or disorders associated with, or characterized by, undesired angiogenesis, but are non-responsive to standard therapies, as well as those who have not previously been treated. The invention also encompasses methods of treating patients regardless of patient's age, although some diseases or disorders are more common in certain age groups. The invention further encompasses methods of treating patients who have undergone surgery in an attempt to treat the disease or condition at issue, as well as those who have not. Because patients with cancer and diseases and disorders characterized by undesired angiogenesis have heterogenous clinical manifestations and varying clinical outcomes, the treatment given to a patient may vary, depending on his/her prognosis. The skilled clinician will be able to readily determine without undue experimentation specific secondary agents, types of surgery, and types of non-drug based standard therapy that can be effectively used to treat an individual patient with cancer and other diseases or disorders.

Methods encompassed by this invention comprise administering one or more immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, to a patient (e.g., a human) suffering, or likely to suffer, from cancer or a disease or disorder mediated by undesired angiogenesis.

In one embodiment of the invention, an immunomodulatory compound of the invention can be administered orally and in single or divided daily doses in an amount of from about 0.10 to about 150 mg/day. In a particular embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of from about 0.1 to about 1 mg per day, or alternatively from about 0.1 to about 5 mg every other day. In a preferred embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered in an amount of from about 5 to 25 mg per day, or alternatively from about 10 to about 50 mg every other day.

In a specific embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of about 1, 2, or 5 mg per day to patients with relapsed multiple myeloma. In a particular embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered initially in an amount of 5 mg/day and the dose can be escalated every week to 10, 20, 25, 30 and 50 mg/day. In a specific embodiment, Revimid™ can be administered in an amount of up to about 30 mg/day to patients with solid tumor. In a particular embodiment, Revimid™ can be administered in an amount of up to about 40 mg/day to patients with glioma.

#### 5.3.1 Combination Therapy with a Second Active Agent

Specific methods of the invention comprise administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with one or more second active agents, and/or in combination with radiation therapy, blood transfusions, or surgery. Examples of immunomodulatory compounds of the invention are disclosed herein (see, e.g., section 5.1). Examples of second active agents are also disclosed herein (see, e.g., section 5.2).

Administration of the immunomodulatory compounds and the second active agents to a patient can occur simultaneously or sequentially by the same or different routes of administration. The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally

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without decomposing prior to entering the blood stream) and the disease being treated. A preferred route of administration for an immunomodulatory compound of the invention is orally. Preferred routes of administration for the second active agents or ingredients of the invention are known to those of ordinary skill in the art. See, e.g., *Physicians' Desk Reference*, 1755-1760 (56<sup>th</sup> ed., 2002).

In one embodiment of the invention, the second active agent is administered intravenously or subcutaneously and once or twice daily in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. The specific amount of the second active agent will depend on the specific agent used, the type of disease being treated or managed, the severity and stage of disease, and the amount(s) of immunomodulatory compounds of the invention and any optional additional active agents concurrently administered to the patient. In a particular embodiment, the second active agent is oblimersen (Genasense®), GM-CSF, G-CSF, EPO, taxotere, irinotecan, dacarbazine, transretinoic acid, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, vincristine, doxorubicin, COX-2 inhibitor, IL2, IL8, IL18, IFN, Ara-C, vinorelbine, or a combination thereof.

In a particular embodiment, GM-CSF, G-CSF or EPO is administered subcutaneously during about five days in a four or six week cycle in an amount of from about 1 to about 750 mg/m<sup>2</sup>/day, preferably in an amount of from about 25 to about 500 mg/m<sup>2</sup>/day, more preferably in an amount of from about 50 to about 250 mg/m<sup>2</sup>/day, and most preferably in an amount of from about 50 to about 200 mg/m<sup>2</sup>/day. In a certain embodiment, GM-CSF may be administered in an amount of from about 60 to about 500 mcg/m<sup>2</sup> intravenously over 2 hours, or from about 5 to about 12 mcg/m<sup>2</sup>/day subcutaneously. In a specific embodiment, G-CSF may be administered subcutaneously in an amount of about 1 mcg/kg/day initially and can be adjusted depending on rise of total granulocyte counts. The maintenance dose of G-CSF may be administered in an amount of about 300 (in smaller patients) or 480 mcg subcutaneously. In a certain embodiment, EPO may be administered subcutaneously in an amount of 10,000 Unit 3 times per week.

In another embodiment, Revimid™ in an amount of about 25 mg/d and dacarbazine in an amount of about from 200 to 1,000 mg/m<sup>2</sup>/d are administered to patients with metastatic malignant melanoma. In a specific embodiment, Revimid™ is administered in an amount of from about 5 to about 25 mg/d to patients with metastatic malignant melanoma whose disease has progressed on treatment with dacarbazine, IL-2 or IFN. In a specific embodiment, Revimid™ is administered to patients with relapsed or refractory multiple myeloma in an amount of about 15 mg/d twice a day or about 30 mg/d four times a day in a combination with dexamethasone.

In another embodiment, an immunomodulatory compound is administered with melphalan and dexamethasone to patients with amyloidosis. In a specific embodiment, an immunomodulatory compound of the invention and steroids can be administered to patients with amyloidosis.

In another embodiment, an immunomodulatory compound is administered with gemcitabine and cisplatin to patients with locally advanced or metastatic transitional cell bladder cancer.

In another embodiment, an immunomodulatory compound is administered in combination with a second active ingredient as follows: temozolomide to pediatric patients with relapsed or progressive brain tumors or recurrent neuroblastoma; celecoxib, etoposide and cyclophosphamide for relapsed or progressive CNS cancer; temodar to patients with

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recurrent or progressive meningioma, malignant meningioma, hemangiopericytoma, multiple brain metastases, relapsed brain tumors, or newly diagnosed glioblastoma multiformis; irinotecan to patients with recurrent glioblastoma; carboplatin to pediatric patients with brain stem glioma; procarbazine to pediatric patients with progressive malignant gliomas; cyclophosphamide to patients with poor prognosis malignant brain tumors, newly diagnosed or recurrent glioblastoma multiformis; Gliadel® for high grade recurrent malignant gliomas; temozolomide and tamoxifen for anaplastic astrocytoma; or topotecan for gliomas, glioblastoma, anaplastic astrocytoma or anaplastic oligodendroglioma.

In another embodiment, an immunomodulatory compound is administered with methotrexate and cyclophosphamide to patients with metastatic breast cancer.

In another embodiment, an immunomodulatory compound is administered with temozolomide to patients with neuroendocrine tumors.

In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with recurrent or metastatic head or neck cancer. In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with pancreatic cancer.

In another embodiment, an immunomodulatory compound is administered to patients with colon cancer in combination with Arisa®, taxol and/or taxotere.

In another embodiment, an immunomodulatory compound is administered with capecitabine to patients with refractory colorectal cancer or patients who fail first line therapy or have poor performance in colon or rectal adenocarcinoma.

In another embodiment, an immunomodulatory compound is administered in combination with fluorouracil, leucovorin, and irinotecan to patients with Dukes C & D colorectal cancer or to patients who have been previously treated for metastatic colorectal cancer.

In another embodiment, an immunomodulatory compound is administered to patients with refractory colorectal cancer in combination with capecitabine, xeloda, and/or CPT-11.

In another embodiment, an immunomodulatory compound of the invention is administered with capecitabine and irinotecan to patients with refractory colorectal cancer or to patients with unresectable or metastatic colorectal carcinoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with interferon alpha or capecitabine to patients with unresectable or metastatic hepatocellular carcinoma; or with cisplatin and thiopeta to patients with primary or metastatic liver cancer.

In another embodiment, an immunomodulatory compound is administered in combination with pegylated interferon alpha to patients with Kaposi's sarcoma.

In another embodiment, an immunomodulatory compound is administered in combination with fludarabine, carboplatin, and/or topotecan to patients with refractory or relapsed or high-risk acute myelogenous leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with liposomal daunorubicin, topotecan and/or cytarabine to patients with unfavorable karyotype acute myeloblastic leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with gemcitabine and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered in combination with carboplatin and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered with dox-



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etaxol to patients with non-small cell lung cancer who have been previously treated with carbo/VP 16 and radiotherapy.

In another embodiment, an immunomodulatory compound is administered in combination with carboplatin and/or taxotere, or in combination with carboplatin, paclitaxel and/or thoracic radiotherapy to patients with non-small cell lung cancer. In a specific embodiment, an immunomodulatory compound is administered in combination with taxotere to patients with stage IIIB or IV non-small cell lung cancer.

In another embodiment, an immunomodulatory compound of the invention is administered in combination with oblimersen (Genasense®) to patients with small cell lung cancer.

In another embodiment, an immunomodulatory compound is administered alone or in combination with a second active ingredient such as vinblastine or fludarabine to patients with various types of lymphoma, including, but not limited to, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma or relapsed or refractory low grade follicular lymphoma.

In another embodiment, an immunomodulatory compound is administered in combination with taxotere, IL-2, IFN, GM-CSF, and/or dacarbazine to patients with various types or stages of melanoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with vinorelbine to patients with malignant mesothelioma, or stage IIIB non-small cell lung cancer with pleural implants or malignant pleural effusion mesothelioma syndrome.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of multiple myeloma in combination with dexamethasone, zoledronic acid, palmitronate, GM-CSF, biacin, vinblastine, melphalan, busulphan, cyclophosphamide, IFN, palmidronate, prednisone, bisphosphonate, celecoxib, arsenic trioxide, PEG INTRON-A, vincristine, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with relapsed or refractory multiple myeloma in combination with doxorubicin (Doxil®), vincristine and/or dexamethasone (Decadron®).

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of ovarian cancer such as peritoneal carcinoma, papillary serous carcinoma, refractory ovarian cancer or recurrent ovarian cancer, in combination with taxol, carboplatin, doxorubicin, gemcitabine, cisplatin, xeloda, paclitaxel, dexamethasone, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of prostate cancer, in combination with xeloda, 5 FU/LV, gemcitabine, irinotecan plus gemcitabine, cyclophosphamide, vincristine, dexamethasone, GM-CSF, celecoxib, taxotere, ganciclovir, paclitaxel, adriamycin, docetaxel, estramustine, Emcyt, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of renal cell cancer, in combination with capecitabine, IFN, tamoxifen, IL-2, GM-CSF, Celebrex®, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of gynecologic, uterus or soft tissue sarcoma cancer in combination with IFN, a COX-2 inhibitor such as Celebrex®, and/or sulindac.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of

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solid tumors in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apicitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with scleroderma or cutaneous vasculitis in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apicitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

This invention also encompasses a method of increasing the dosage of an anti-cancer drug or agent that can be safely and effectively administered to a patient, which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable derivative, salt, solvate, clathrate, hydrate, or pro-drug thereof. Patients that can benefit by this method are those likely to suffer from an adverse effect associated with anti-cancer drugs for treating a specific cancer of the skin, subcutaneous tissue, lymph nodes, brain, lung, liver, bone, intestine, colon, heart, pancreas, adrenal, kidney, prostate, breast, colorectal, or combinations thereof. The administration of an immunomodulatory compound of the invention alleviates or reduces adverse effects which are of such severity that it would otherwise limit the amount of anti-cancer drug.

In one embodiment, an immunomodulatory compound of the invention can be administered orally and daily in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 50 mg, more preferably from about 2 to about 25 mg prior to, during, or after the occurrence of the adverse effect associated with the administration of an anti-cancer drug to a patient. In a particular embodiment, an immunomodulatory compound of the invention is administered in combination with specific agents such as heparin, aspirin, coumadin, or G-CSF to avoid adverse effects that are associated with anti-cancer drugs such as but not limited to neutropenia or thrombocytopenia.

In one embodiment, an immunomodulatory compound of the invention can be administered to patients with diseases and disorders associated with, or characterized by, undesired angiogenesis in combination with additional active ingredients including but not limited to anti-cancer drugs, anti-inflammatories, antihistamines, antibiotics, and steroids.

In another embodiment, this invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with (e.g. before, during, or after) conventional therapy including, but not limited to, surgery, immunotherapy, biological therapy, radiation therapy, or other non-drug based therapy presently used to treat, prevent or manage cancer. The combined use of the immunomodulatory compounds of the invention and conventional therapy may provide a unique treatment regimen that is unexpectedly effective in certain patients. Without being limited by theory, it is believed that immunomodulatory compounds of the invention may provide additive or synergistic effects when given concurrently with conventional therapy.

As discussed elsewhere herein, the invention encompasses a method of reducing, treating and/or preventing adverse or undesired effects associated with conventional therapy including, but not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy. One or more immunomodulatory compounds of the invention and other active ingredient can be administered to a patient prior to, during, or after the occurrence of the adverse effect associated with conventional therapy.

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In one embodiment, an immunomodulatory compound of the invention can be administered in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 25 mg, more preferably from about 2 to about 10 mg orally and daily alone, or in combination with a second active agent disclosed herein (see, e.g., section 5.2), prior to, during, or after the use of conventional therapy.

In a specific embodiment of this method, an immunomodulatory compound of the invention and doxorubicin are administered to patients with non-small cell lung cancer who were previously treated with carboplatin and radiotherapy.

#### 5.3.2 Use with Transplantation Therapy

Compounds of the invention can be used to reduce the risk of Graft Versus Host Disease (GVHD). Therefore, the invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering the immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with transplantation therapy.

As those of ordinary skill in the art are aware, the treatment of cancer is often based on the stages and mechanism of the disease. For example, as inevitable leukemic transformation develops in certain stages of cancer, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. The combined use of the immunomodulatory compound of the invention and transplantation therapy provides a unique and unexpected synergism. In particular, an immunomodulatory compound of the invention exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy in patients with cancer.

An immunomodulatory compound of the invention can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of GVHD. This invention encompasses a method of treating, preventing and/or managing cancer which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after the transplantation of umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow. Examples of stem cells suitable for use in the methods of the invention are disclosed in U.S. provisional patent application No. 60/372,348, filed Apr. 12, 2002 by R. Hariri et al., the entirety of which is incorporated herein by reference.

In one embodiment of this method, an immunomodulatory compound of the invention is administered to patients with multiple myeloma before, during, or after the transplantation of autologous peripheral blood progenitor cell.

In another embodiment, an immunomodulatory compound is administered to patients with relapsing multiple myeloma after the stem cell transplantation.

In another embodiment, an immunomodulatory compound and prednisone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous stem cell.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as salvage therapy for low risk post transplantation to patients with multiple myeloma.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous bone marrow.

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In another embodiment, an immunomodulatory compound is administered following the administration of high dose of melphalan and the transplantation of autologous stem cell to patients with chemotherapy responsive multiple myeloma.

In another embodiment, an immunomodulatory compound and PEG INTRO-A are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous CD34-selected peripheral stem cell.

In another embodiment, an immunomodulatory compound is administered with post transplant consolidation chemotherapy to patients with newly diagnosed multiple myeloma to evaluate anti-angiogenesis.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy after DCEP consolidation, following the treatment with high dose of melphalan and the transplantation of peripheral blood stem cell to 65 years of age or older patients with multiple myeloma.

#### 5.3.3 Cycling Therapy

In certain embodiments, the prophylactic or therapeutic agents of the invention are cyclically administered to a patient. Cycling therapy involves the administration of an active agent for a period of time, followed by a rest for a period of time, and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

Consequently, in one specific embodiment of the invention, an immunomodulatory compound of the invention is administered daily in a single or divided doses in a four to six week cycle with a rest period of about a week or two weeks. The invention further allows the frequency, number, and length of dosing cycles to be increased. Thus, another specific embodiment of the invention encompasses the administration of an immunomodulatory compound of the invention for more cycles than are typical when it is administered alone. In yet another specific embodiment of the invention, an immunomodulatory compound of the invention is administered for a greater number of cycles that would typically cause dose-limiting toxicity in a patient to whom a second active ingredient is not also being administered.

In one embodiment, an immunomodulatory compound of the invention is administered daily and continuously for three or four weeks at a dose of from about 0.1 to about 150 mg/d followed by a break of one or two weeks. Actimid™ is preferably administered daily and continuously at an initial dose of 0.1 to 5 mg/d with dose escalation (every week) by 1 to 10 mg/d to a maximum dose of 50 mg/d for as long as therapy is tolerated. In a particular embodiment, Revimid™ is administered in an amount of about 5, 10, or 25 mg/day, preferably in an amount of about 10 mg/day for three to four weeks, followed by one week or two weeks of rest in a four or six week cycle.

In one embodiment of the invention, an immunomodulatory compound of the invention and a second active ingredient are administered orally, with administration of an immunomodulatory compound of the invention occurring 30 to 60 minutes prior to a second active ingredient, during a cycle of four to six weeks. In another embodiment of the invention, the combination of an immunomodulatory compound of the invention and a second active ingredient is administered by intravenous infusion over about 90 minutes every cycle. In a specific embodiment, one cycle comprises the administration of from about 10 to about 25 mg/day of Revimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient daily for three to four weeks and then one or two weeks of



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rest. In another specific embodiment, each cycle comprises the administration of from about 5 to about 10 mg/day of Actimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient for 3 to 4 weeks followed by one or two weeks of rest. Typically, the number of cycles during which the combinatorial treatment is administered to a patient will be from about one to about 24 cycles, more typically from about two to about 16 cycles, and even more typically from about four to about three cycles.

#### 5.4 Pharmaceutical Compositions and Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Pharmaceutical compositions and dosage forms of the invention can further comprise one or more excipients.

Pharmaceutical compositions and dosage forms of the invention can also comprise one or more additional active ingredients. Consequently, pharmaceutical compositions and dosage forms of the invention comprise the active ingredients disclosed herein (e.g., an immunomodulatory compound and a second active agent). Examples of optional second, or additional, active ingredients are disclosed herein (see, e.g., section 5.2).

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), topical (e.g., eye drops or other ophthalmic preparations), transdermal or transcutaneous administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; eye drops or other ophthalmic preparations suitable for topical administration; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as

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tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients may be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose other mono- or disaccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the *U.S. Pharmacopeia* (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise an immunomodulatory compound of the invention or a pharmaceutically

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acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of from about 0.10 to about 150 mg. Typical dosage forms comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of about 0.1, 1, 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 50, 100, 150 or 200 mg. In a particular embodiment, a preferred dosage form comprises 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) in an amount of about 1, 2, 5, 10, 25 or 50 mg. In a specific embodiment, a preferred dosage form comprises 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) in an amount of about 5, 10, 25 or 50 mg. Typical dosage forms comprise the second active ingredient in an amount of 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. Of course, the specific amount of the anti-cancer drug will depend on the specific agent used, the type of cancer being treated or managed, and the amount(s) of an immunomodulatory compound of the invention and any optional additional active agents concurrently administered to the patient.

#### 5.4.1 Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other

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starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, PA), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

A preferred solid oral dosage form of the invention comprises an immunomodulatory compound of the invention,

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anhydrous lactose, microcrystalline cellulose, polyvinylpyrrolidone, stearic acid, colloidal anhydrous silica, and gelatin.

#### 5.4.2 Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845, 770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674, 533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

#### 5.4.3 Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose

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Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention. For example, cyclodextrin and its derivatives can be used to increase the solubility of an immunomodulatory compound of the invention and its derivatives. See, e.g., U.S. Pat. No. 5,134,127, which is incorporated herein by reference.

#### 5.4.4 Topical and Mucosal Dosage Forms

Topical and mucosal dosage forms of the invention include, but are not limited to, sprays, aerosols, solutions, emulsions, suspensions, eye drops or other ophthalmic preparations, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide topical and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form solutions, emulsions or gels, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990).

The pH of a pharmaceutical composition or dosage form may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

#### 5.4.5 Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a dosage form of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt salt, solvate, hydrate, stereoisomer, prodrug, or clathrate thereof. Kits encompassed by this invention can further comprise additional active ingredients such as oblimersen (Genasense®), melphalan, G-CSF, GM-CSF, EPO, topotecan, dacarbazine, irinotecan, taxotere, IFN,



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COX-2 inhibitor, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13 cis-retinoic acid, or a pharmacologically active mutant or derivative thereof, or a combination thereof. Examples of the additional active ingredients include, but are not limited to, those disclosed herein (see, e.g., section 5.2).

Kits of the invention can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

## 6. EXAMPLES

Certain embodiments of the invention are illustrated by the following non-limiting examples.

### 6.1 Modulation of Cytokine Production

A series of non-clinical pharmacology and toxicology studies have been performed to support the clinical evaluation of an immunomodulatory compound of the invention in human subjects. These studies were performed in accordance with internationally recognized guidelines for study design and in compliance with the requirements of Good Laboratory Practice (GLP), unless otherwise noted.

Inhibition of TNF- $\alpha$  production following LPS-stimulation of human PBMC and human whole blood by 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Ac-timid<sup>TM</sup>), 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione and thalidomide (Revimid<sup>TM</sup>) was investigated in vitro (Muller et al., *Bioorg. Med. Chem. Lett.* 9:1625-1630, 1999). The IC<sub>50</sub>'s of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and human whole blood were ~24 nM (6.55 ng/mL) and ~25 nM (6.83 ng/mL), respectively. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but at least 200 times more potent than, thalidomide. In vitro studies have also demonstrated that concentrations of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione of 2.73 to 27.3 ng/mL (0.01 to 0.1  $\mu$ M) achieved 50% inhibition of the proliferation of MM.1S and Hs Sultan cells.

The IC<sub>50</sub>'s of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and human whole blood were ~100 nM (25.9 ng/mL) and ~480 nM (103.6 ng/mL), respectively. Thalidomide, in contrast, had an IC<sub>50</sub> of ~194  $\mu$ M (50.2  $\mu$ g/mL) for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-

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dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but 50 to 2000 times more potent than, thalidomide. It has been shown that the compound is approximately 50-100 times more potent than thalidomide in stimulating the proliferation of T-cells following primary induction by T-cell receptor (TCR) activation. 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is also approximately 50 to 100 times more potent than thalidomide in augmenting the production of IL-2 and IFN- $\gamma$  following TCR activation of PBMC (IL-2) or T-cells (IFN- $\gamma$ ). In addition, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione exhibited dose-dependent inhibition of LPS-stimulated production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 by PBMC while it increased production of the anti-inflammatory cytokine IL-10.

### 6.2 Inhibition of MM Cell Proliferation

The ability of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid<sup>TM</sup>) and thalidomide for comparison to effect the proliferation of MM cell lines has been investigated in an in vitro study. Uptake [<sup>3</sup>H]-thymidine by different MM cell lines (MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of cell proliferation. Cells were incubated in the presence of compounds for 48 hours; [<sup>3</sup>H]-thymidine was included for the last 8 hours of the incubation period. Addition of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione to MM. 1S and Hs Sultan cells resulted in 50% inhibition of cell proliferation at concentrations of 0.4  $\mu$ M and 1  $\mu$ M, respectively. In contrast, addition of thalidomide at concentrations up to 100  $\mu$ M resulted in only 15% and 20% inhibition of cell proliferation in MM.1S and Hs Sultan cells, respectively. These data are summarized in FIG. 1.

### 6.3 Toxicology Studies

The effects of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid<sup>TM</sup>) on cardiovascular and respiratory function are investigated in anesthetized dogs. Two groups of Beagle dogs (2/sex/group) are used. One group receives three doses of vehicle only and the other receives three ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (2, 10, and 20 mg/kg). In all cases, doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione or vehicle are successively administered via infusion through the jugular vein separated by intervals of at least 30 minutes.

The cardiovascular and respiratory changes induced by 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione are minimal at all doses when compared to the vehicle control group. The only statistically significant difference between the vehicle and treatment groups is a small increase in arterial blood pressure (from 94 mmHg to 101 mmHg) following administration of the low dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. This effect lasts approximately 15 minutes and is not seen at higher doses. Deviations in femoral blood flow, respiratory parameters, and Qtc interval are common to both the control and treated groups and are not considered treatment-related.

### 6.4 Cycling Therapy in Patients

In a specific embodiment, an immunomodulatory compound of the invention are cyclically administered to patients with cancer. Cycling therapy involves the administration of a first agent for a period of time, followed by a rest for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

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In a specific embodiment, prophylactic or therapeutic agents are administered in a cycle of about 4 to 6 weeks, about once or twice every day. One cycle can comprise the administration of a therapeutic or prophylactic agent for three to four weeks and at least a week or two weeks of rest. The number of cycles administered is from about one to about 24 cycles, more typically from about two to about 16 cycles, and more typically from about four to about eight cycles.

For example, in a cycle of four weeks, on day 1, the administration of 25 mg/d of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is started. On day 22, the administration of the compound is stopped for a week of rest. On day 29, the administration of 25 mg/d 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is begun.

#### 6.5 Clinical Studies in Patients

##### 6.5.1 Treatment of Relapsed Multiple Myeloma

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) was administered to patients with relapsed/refractory multiple myeloma. The study was conducted in compliance with Good Clinical Practices. Patients were at least 18 years old, had been diagnosed with multiple myeloma (with paraprotein in serum and/or urine), and were considered refractory to treatment after at least two cycles of treatment, or have relapsed after two cycles of treatment.

Patients who have progressive disease, according to the Southwest Oncology Group (SWOG) criteria, on their prior regimen are considered treatment refractory. Relapse following remission is defined as >25% increase in M component from baseline levels; reappearance of the M paraprotein that had previously disappeared; or a definite increase in the size and number of lytic bone lesions recognized on radiographs. Patients may have had prior therapy with thalidomide, provided they were able to tolerate the treatment. A Zubrod performance status of 0 to 2 is required for all patients.

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione is administered to patients at doses of 1, 2, 5, or 10 mg/day for up to four weeks; at each dose level, three patients are initially enrolled. Dosing occurs at approximately the same time each morning; all doses are administered in the fasted state (no eating for at least two hours prior to dosing and two hours after dosing). 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione doses are administered in an ascending fashion such that patients in the first cohort receive the lowest dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (1 mg/day) and escalation to the next higher dose level occurs only following the establishment of safety and tolerability at the current dose. If one out of three patients at any dose level experience dose limiting toxicity (DLT), three

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additional patients are enrolled at that dose. If none of the three additional patients experience DLT, escalation to the next dose level occurs; dose escalations continue in a similar fashion until the MTD is established or the maximum daily dose (10 mg/day) is attained. However, if one of the three additional patients enrolled experiences DLT, the MTD has been reached. If two or more of the three additional patients enrolled experience DLT, the MTD is judged to have been exceeded and three additional patients are enrolled at the preceding dose level to confirm the MTD. Once the MTD has been identified, four additional patients are enrolled at that dose level so that a total of 10 patients is treated at the MTD.

Blood sampling for analysis of pharmacokinetic parameters is performed on Days 1 and 28 according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. An additional blood sample is collected at each weekly visit for the determination of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione levels. Total urine collections are also made with urine pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Safety assessments are made by monitoring adverse events, vital signs, ECGs, clinical laboratory evaluations (blood chemistry, hematology, lymphocyte phenotyping, and urinalysis), and physical examination at specific times during the study.

Results of interim pharmacokinetic analyses obtained following single- and multiple-dose administration of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione to multiple myeloma patients are presented below in Tables 1 and 2. These data show that 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione was steadily absorbed at all dose levels in relapsed multiple myeloma patients. Maximum plasma concentrations occurred at a median  $T_{max}$  of between 2.5 and 2.8 hours post-dose at Day 1 and between 3 and 4 hours post-dose at Week 4. At all doses, plasma concentrations declined in a monophasic manner after reaching  $C_{max}$ . The start of the elimination phase occurred between 3 and 10 hours post-dose at Day 1 and Week 4, respectively.

These data also showed that after 4 weeks of dosing, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione accumulated to a small extent (mean accumulation ratios ~1.02 to 1.52 and ~0.94 to 1.62 for  $C_{max}$  and  $AUC_{(0-\infty)}$ , respectively). There was almost a dose proportional increase in  $AUC_{(0-\infty)}$  and  $C_{max}$  values with increasing dose. A five-fold higher dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione produced a 3.2- and 2.2-fold increase in  $C_{max}$  at Day 1 and Week 4, respectively. Similarly, a 5-fold increase in dose resulted in a 3.6- and 2.3-fold increase in  $AUC_{(0-\infty)}$ , at Day 1 and Week 4, respectively.

TABLE 1

Pharmacokinetic parameters of Actimid™ in relapsed multiple myeloma patients				
Parameter		1 mg (N = 6)	2 mg (N = 2)	5 mg (N = 3)
		Day 1		
$C_{max}$	ng/mL	15.03 (4.04)	24.4* (12.1)	48.56 (14.03)
$t_{max}$	h	3.3 (2.6)	2.7* (0.3)	2.3 (0.3)
$AUC_{(0-\infty)}$	ng.h/mL	152.90 (36.62)	279.18 (51.10)	593.10 (335.23)
$AUC_{(0-\tau)}$		134.21 (27.14)	249.57 (29.26)	520.94 (267.32)
$t_{1/2}$	h	7.3 (3.4)	6.3 (1.4)	6.5 (2.2)
CL/F	mL/min	114.75 (29.20)	121.43 (22.22)	182.31 (117.06)
Vz/f	L	69.55 (44.97)	65.31 (2.80)	87.24 (22.61)

t = 24 hours

N/A = not available

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TABLE 2

Pharmacokinetic parameters of Actimid™ following multiple oral doses (1, 2, and 5 mg/day) in relapsed multiple myeloma patients				
Parameter		1 mg (N = 5)	2 mg (N = 2)	5 mg (N = 3)
		Week 4		
$C_{max}$	ng/mL	23.20 (7.48)	30.05* (15.64)	58.07 (38.08)
$t_{max}$	h	3.6 (1.5)	2.8* (0.3)	5.0 (2.6)
$AUC_{(0-\infty)}$	ng.h/mL	N/A	N/A	N/A
$AUC_{(0-\tau)}$		239.31 (122.59)	269.36 (186.34)	597.24 (354.23)
$t_{1/2}$	h	6.2* (0.6)	7.7 (2.8)	7.8 (4.0)
CL/F	mL/min	87.85 (48.48)	162.68 (112.54)	207.50 (175.41)
Vz/f	L	41.35* (8.84)	95.04 (35.39)	103.95 (27.25)

 $\tau$  = 24 hours

N/A = not available

\*N = 3 patients

### 6.5.2 Treatment of Relapsed Multiple Myeloma

Two Phase 1 clinical studies of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) have been conducted to identify the maximum tolerated dose (MTD) in patients with refractory or relapsed multiple myeloma. These studies have also characterized the safety profile of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione when ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione were given orally for up to 4 weeks. Patients started 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment at 5 mg/day with subsequent escalation to 10, 25, and 50 mg/day. Patients were enrolled for 28 days at their assigned dose, with the option of extended treatment for those who did not exhibit disease progression or experience dose limiting toxicity (DLT). Patients were evaluated for adverse events at each visit and the severity of these events was graded according to the National Cancer Institute (NCI) Common Toxicity Criteria. Patients were discontinued if they experienced DLT (Grade 3 or greater non-hematological, or Grade 4 hematological toxicity).

In this study, 27 patients were enrolled. All patients had relapsed multiple myeloma and 18 (72%) were refractory to salvage therapy. Among these patients, 15 had undergone prior autologous stem cell transplantation and 16 patients had received prior thalidomide treatment. The median number of prior regimens was 3 (range 2 to 6).

Blood and urine samples were collected for analysis of pharmacokinetic parameters on Days 1 and 28. Blood samples were collected according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. In addition, a blood sample was collected at each weekly clinic visit for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione determination. Total urine was collected and pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Response to treatment was assessed by M-protein quantification (by immunoelectrophoresis) from serum and a 24-hour urine collection, with creatinine clearance and 24-hour protein calculations undertaken at screening, baseline, Weeks 2 and 4, and monthly thereafter (or upon early termination). Bone marrow aspirations and/or tissue biopsy are also performed at Months 3, 6 and 12 if a patient's paraprotein serum concentration or 24-hour urine protein excretion declined to the next lower level, based on best response criteria. Preliminary results for the 28-day treatment period are summarized below.

Preliminary pharmacokinetic analyses based on these two studies indicated that AUC and  $C_{max}$  values increase propor-

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tionally with dose following single and multiple doses in multiple myeloma patients (as was seen in healthy volunteers). Further, there was no evidence of accumulation with multiple dosing as single dose  $AUC_{(0-\infty)}$  was comparable to multiple dose  $AUC_{(0-\tau)}$  following the same dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Similar to healthy volunteer studies, double peaks were observed. Exposure in multiple myeloma patients appeared to be slightly higher based on  $C_{max}$  and AUC values as compared to healthy male volunteers while clearance in multiple myeloma patients was lower than it was in healthy volunteers, consistent with their poorer renal function (both as a consequence of their age and their disease). Finally, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione half-life in patients was shorter than in healthy volunteers (mean 8 hours, ranging up to 17 hours).

In this study, the first cohort of 3 patients was treated for 28 days at 5 mg/day without any dose limiting toxicity (DLT). The second cohort of 3 patients subsequently commenced therapy at 10 mg/day. Patients in the second 10 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione cohort tolerated treatment well.

### 6.5.3 Treatment of Solid Tumors

Study with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) was conducted in patients with varying types of solid tumors, including malignant melanoma (13), carcinoma of the pancreas (2), carcinoid-unknown primary (1), renal carcinoma (1), breast carcinoma (1) and NSCLC (2). Patients received 5 mg/day 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for seven days and are subsequently escalated every seven days to 10 mg/day, 25 mg/day, and 50 mg/day for a total of 4 weeks of treatment. Patients who, experienced clinical benefit were permitted to continue on treatment as Named Patients.

The study initially enrolled 20 patients and was subsequently amended to enroll 16 additional patients (adrenal carcinoma, NSCLC, malignant mesothelioma, breast cancer, malignant melanoma (8), renal cell cancer (4)) at a higher dose. The 16 additional patients were given weekly escalating doses of 25 mg/day, 50 mg/day, 75 mg/day, 100 mg/day, 125 mg/day, and 150 mg/day over a 6-week period with continuing treatment for an additional six weeks.

The study of Phase 1 study was designed to determine a maximum tolerated dose (MTD) of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in patients with refractory solid tumors and/or lymphoma, as well as to characterize the pharmacokinetic and side effect profiles of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in this patient population. The study design dictates that

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at least 3 patients must be enrolled at a dose level and have completed 28 days of treatment prior to enrollment of patients at the next higher dose level. Patients in the first cohort began dosing at 5 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Patients will be escalated to 10, 20, 25, and 30 mg/day provided there is no toxicity.

In this study, the MTD is defined as the highest dose level in which fewer than two of six patients treated did not experience Grade 3 or greater non-hematological toxicity or Grade 4 or greater hematological toxicity. If, at any given dose level in either study, one out of three patients experiences toxicity, three additional patients must be treated at that particular dose. If, however, two out of six patients experience DLT, the MTD is judged to have been exceeded. No further dose escalations are to occur and additional patients are to be enrolled at the previous dose level. The dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered is escalated until the MTD is achieved or the maximum daily dose of is reached.

No DLTs were reported in the initial group of 20 patients enrolled in the study. Thirteen of the original 20 trial patients, along with 2 non-trial patients, continued on treatment as named patients at doses up to 150 mg/day.

#### 6.5.4 Treatment of Gliomas

This study was performed to find toxicity in patients with recurrent, high-grade gliomas. The study is designed such that patients are given increasingly higher doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione until a maximum tolerated dose (MTD) is established. The study also seeks to obtain preliminary toxicity information and pharmacokinetic data on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, as well as to develop exploratory data concerning surrogate end points of angiogenic activity in vivo using functional neuro-imaging studies, and in vitro assays of serum angiogenic peptides.

Patients enrolled in the first cohort receive 2.5 mg/m<sup>2</sup>/day for a 4-week cycle. During each 4-week cycle of therapy, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered once daily for 3 weeks followed by a week of rest. Patients who complete a treatment cycle may receive another cycle of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment if two criteria are met. First, the patient must have stable disease or have experienced a partial response or complete response, or the patient is benefiting from the therapy with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione as evidenced by a decrease in tumor-related symptoms such as neurological deficits. Second, the patient must have recovered from toxicity related to 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione which occurred in the prior cycle by Day 42 or sooner (28-day cycle plus limit of 2 weeks to recover) as evidenced by a return to Grade  $\leq$  1 toxicity level. Patients who experience DLT in the previous cycle should have their dose modified. DLT is defined as a non-hematological event Grade  $\geq$  3 toxicity or hematological event of Grade 4 toxicity thought to be related to the study medication. Patients who experience DLT in the first cycle and have no response to therapy are removed from the study.

3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione doses are subsequently escalated to 5, 8, 11, 15, and 20 mg/m<sup>2</sup>/day to a maximum total daily dose of 40 mg. Patients continue to receive 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione on a 4-week cycle per dose level until one of the off-study criteria are met.

Three patients are enrolled in each cohort. If at least one DLT occurs, three additional patients are added to the cohort at that particular dose level. If two DLTs occur, the MTD,

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defined as the dose at which fewer than one-third of patients at each dose level experiences DLT has been exceeded and four more patients are treated at the previous dose.

Patients who experience DLT during the first 4-week cycle are removed from the study, except if they have a response to therapy. For patients who have completed their first 4-week cycle of without DLT, but who subsequently experience Grade 3 or 4 hematological and/or nonhematological toxicity, treatment is suspended for a minimum of a week. If the toxicity resolves to  $<$ Grade 2 within three weeks, the patient is treated at two dose levels lower than the dose that caused the toxicity (or a 50% reduction if the patient was treated at the first or second dose level). Patients in whom Grade 3 or 4 toxicity does not resolve to  $<$ Grade 1 within three weeks, or those who have another Grade 3 toxicity at the reduced dose are removed from the study.

Pharmacokinetic sampling is performed prior the first dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Day 1) and 0.5, 1, 2, 4, 6, 8, 24, and 48 hours thereafter. Sampling is also conducted pre-dose on Days 7 and 21 and 0.5, 1, 2, 4, 6, 8, and 24 post-dose on Day 21 to evaluate steady-state 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione levels.

#### 6.5.5 Treatment of Metastatic Melanoma

Patients with metastatic melanoma were started on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revmid™) at 5 mg/day for seven days. The dose was then increased every seven days to 10 mg/day, 25 mg/day, and 50 mg/day, respectively, for a total of four weeks on therapy. Five of the 13 melanoma patients who were treated under this regimen either showed disease stabilization or a partial response in the first four weeks of treatment. Tumor response was seen in cutaneous and subcutaneous lesions (five patients), lymph nodes (two patients), and liver (one patient). The duration of response was approximately six months. The result suggests that the compound appears is a promising new anti-cancer agent and has both antiangiogenic and immunomodulatory properties.

#### 6.5.6 Treatment of Relapsed or Refractory Multiple Myeloma

Patients with relapsed and refractory Dune-Salmon stage III multiple myeloma, who have either failed at least three previous regimens or presented with poor performance status, neutropenia or thrombocytopenia, are treated with up to four cycles of combination of melphalan (50 mg intravenously), an immunomodulatory compound of the invention (about 1 to 150 mg orally daily), and dexamethasone (40 mg/day orally on days 1 to 4) every four to six weeks. Maintenance treatment consisting of daily an immunomodulatory compound of the invention and monthly dexamethasone are continued until the disease progression. The therapy using an immunomodulatory compound of the invention in combination with melphalan and dexamethasone is highly active and generally tolerated in heavily pretreated multiple myeloma patients whose prognosis is otherwise poor.

The embodiments of the invention described above are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials, and procedures. All such equivalents are considered to be within the scope of the invention and are encompassed by the appended claims.

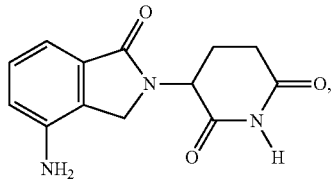
What is claimed is:

1. A method of treating multiple myeloma, which comprises cyclically administering to a patient having multiple myeloma about 5 to about 25 mg per day of a compound of the formula:



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or a pharmaceutically acceptable salt thereof, for 21 consecutive days followed by seven in combination with 40 mg per day dexamethasone.

2. The method of claim 1, wherein the multiple myeloma is smoldering myeloma, indolent myeloma, chemotherapy responsive multiple myeloma, refractory myeloma, relapsed myeloma, or relapsed and refractory Dune-Salmon stage III multiple myeloma.

3. The method of claim 1, wherein the compound is a pharmaceutically acceptable salt.

4. The method of claim 1, wherein the multiple myeloma is relapsed, refractory or resistant to previous therapy.

5. The method of claim 1, wherein the compound and dexamethasone are administered orally.

6. The method of claim 5, wherein the compound is administered in the form of a capsule or tablet.

7. The method of claim 1, wherein the compound is administered in an amount of from about 10 to about 25 mg per day.

8. The method of claim 1, wherein the compound is administered in an amount of about 5, 10, 20, or 25 mg per day.

9. The method of claim 7, wherein the compound is administered in an amount of about 25 mg per day.

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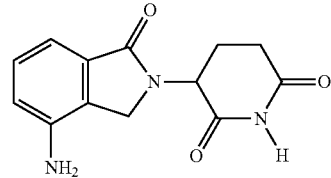
10. The method of claim 1, wherein the compound is administered in an amount of 5 mg per day.

11. The method of claim 7, wherein the compound is administered in an amount of 10 mg per day.

12. The method of claim 1, wherein the compound is administered in a capsule of 5 mg, 10 mg, 15 mg or 25 mg.

13. A method of treating multiple myeloma, which comprises administering, on a 28 day cycle, to a patient having multiple myeloma:

(a) about 25 mg per day of a compound of the formula:



or a pharmaceutically acceptable salt thereof, for 21 consecutive days followed by seven consecutive days of rest from administration of said compound, and; (b) 40 mg per day of dexamethasone on days 1-4 every 28 days.

14. The method of claim 12, wherein the capsule comprises the compound, lactose anhydrous, microcrystalline cellulose, croscarmellose sodium and magnesium stearate.

15. The method of claim 1, wherein said dexamethasone is administered.

\* \* \* \* \*



UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 7,968,569 B2  
APPLICATION NO. : 10/438213  
DATED : June 28, 2011  
INVENTOR(S) : Jerome B. Zeldis

Page 1 of 1

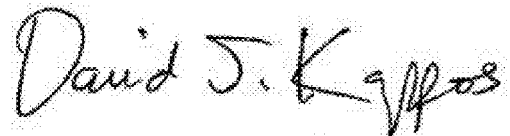
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In claim 1, at column 39, line 11, after “followed by seven”, insert -- consecutive days of rest from administration of said compound during a 28 day cycle, --

In claim 1, at column 39, line 12, between “day” and “dexamethasone”, insert -- of --

In claim 15, at column 40, line 30, after “administered”, insert -- orally --

Signed and Sealed this  
Ninth Day of August, 2011

A handwritten signature in black ink that reads "David J. Kappos". The signature is written in a cursive style with a large, stylized "K" and a long horizontal stroke at the end.

David J. Kappos  
*Director of the United States Patent and Trademark Office*

# **EXHIBIT E**



US008404717B2

(12) **United States Patent**  
**Zeldis**

(10) **Patent No.:** **US 8,404,717 B2**  
(45) **Date of Patent:** **\*Mar. 26, 2013**

(54) **METHODS OF TREATING  
MYELODYSPLASTIC SYNDROMES USING  
LENALIDOMIDE**

(75) Inventor: **Jerome B. Zeldis**, Princeton, NJ (US)

(73) Assignee: **Celgene Corporation**, Summit, NJ (US)

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/070,761**

(22) Filed: **Mar. 24, 2011**

(65) **Prior Publication Data**

US 2011/0172273 A1 Jul. 14, 2011

#### **Related U.S. Application Data**

(60) Continuation of application No. 12/777,765, filed on May 11, 2010, which is a continuation-in-part of application No. 11/985,032, filed on Nov. 12, 2007, now Pat. No. 7,863,297, which is a continuation of application No. 11/654,550, filed on Jan. 16, 2007,

(Continued)

(51) **Int. Cl.**

**A61K 31/47** (2006.01)

**A61K 31/445** (2006.01)

(52) **U.S. Cl.** ..... **514/323; 514/58; 514/319; 514/327**

(58) **Field of Classification Search** ..... **514/323, 514/58, 319, 327**

See application file for complete search history.

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*Primary Examiner* — Michael G. Hartley

*Assistant Examiner* — Jagadishwar Samala

(74) *Attorney, Agent, or Firm* — Jones Day

(57) **ABSTRACT**

Methods of treating, preventing and/or managing myelodysplastic syndromes are disclosed. Specific methods encompass the administrations of 3-(4-amino-1-oxo-1,3-dihydroisoindol-2-yl)-piperidin-2,6-dione in combination with 5-azacytidine.

**10 Claims, No Drawings**

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## Related U.S. Application Data

now Pat. No. 7,393,863, which is a division of application No. 10/411,649, filed on Apr. 11, 2003, now Pat. No. 7,189,740.

- (60) Provisional application No. 60/418,468, filed on Oct. 15, 2002.

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## METHODS OF TREATING MYELOYDYSPLASTIC SYNDROMES USING LENALIDOMIDE

### 1. CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of U.S. patent application Ser. No. 12/777,765, filed May 11, 2010, which is a continuation-in-part of U.S. patent application Ser. No. 11/985,032, filed Nov. 12, 2007, now U.S. Pat. No. 7,863, 297 which is continuation of U.S. patent application Ser. No. 11/654,550 filed Jan. 16, 2007, now issued U.S. Pat. No. 7,393,863, which is divisional of U.S. patent application Ser. No. 10/411,649 filed Apr. 11, 2003, now issued U.S. Pat. No. 7,189,740, which claims the benefit of U.S. Provisional Patent Application No. 60/418,468 filed on Oct. 15, 2002, the contents of each of which are incorporated by reference herein in their entireties.

### 2. FIELD

Provided herein are methods for the treatment of myelodysplastic syndromes ("MDS"), with an immunomodulatory compound, including, but not limited to, 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione, which is also known as lenalidomide or Revlimid®, in combination with a cytidine analog, including, but not limited to, 5-azacytidine. Also included are methods for improving the overall survival of certain classes of patients having MDS. The invention also encompasses pharmaceutical compositions, dosing regimens, and the use of an immunomodulatory compound and 5-azacytidine in conjunction with transplantation therapy and/or other standard therapies for myelodysplastic syndromes.

### 3. BACKGROUND

#### 3.1. Pathobiology of MDS

Myelodysplastic syndrome ("MDS") refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. *The Merck Manual* 953 (17<sup>th</sup> ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424.

The initial hematopoietic stem cell injury can be from causes such as, but not limited to, cytotoxic chemotherapy, radiation, virus, chemical exposure, and genetic predisposition. A clonal mutation predominates over bone marrow, suppressing healthy stem cells. In the early stages of MDS, the main cause of cytopenias is increased programmed cell death (apoptosis). As the disease progresses and converts into leukemia, gene mutation rarely occurs and a proliferation of leukemic cells overwhelms the healthy marrow. The disease course differs, with some cases behaving as an indolent disease and others behaving aggressively with a very short clinical course that converts into an acute form of leukemia.

About 15,000 people are diagnosed with MDS each year in the United States, and about 60,000 are living with the disease. MDS was first considered a distinct disease in 1976, and occurrence was estimated at 1500 new cases every year. At that time, only patients with less than five percent blasts were considered to have this disorder. Statistics from 1999 estimated 13,000 new cases per year and about 1000 cases per year in children, surpassing chronic lymphocytic leukemia as

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the most common form of leukemia in the western hemisphere. The perception that the incidence is increasing may be due to improvements in recognition and criteria for diagnosis. The disease is found worldwide.

5 An international group of hematologists, the French-American-British (FAB) Cooperative Group, classified MDS disorders into five subgroups, differentiating them from acute myeloid leukemia. *The Merck Manual* 954 (17<sup>th</sup> ed. 1999); Bennett J. M., et al., *Ann. Intern. Med.* 1985 October, 103(4): 620-5; and Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599-617. An underlying trilineage dysplastic change in the bone marrow cells of the patients is found in all subtypes.

There are two subgroups of refractory anemia characterized by five percent or less myeloblasts in bone marrow: (1) refractory anemia (RA) and; (2) RA with ringed sideroblasts (BARS), defined morphologically as having 15% erythroid cells with abnormal ringed sideroblasts, reflecting an abnormal iron accumulation in the mitochondria. Both have a prolonged clinical course and low incidence of progression to acute leukemia. Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599-617.

There are two subgroups of refractory anemias with greater than five percent myeloblasts: (1) RA with excess blasts (RAEB), defined as 6-20% myeloblasts, and (2) RAEB in transformation (RAEB-T), with 21-30% myeloblasts. The higher the percentage of myeloblasts, the shorter the clinical course and the closer the disease is to acute myelogenous leukemia. Patient transition from early to more advanced stages indicates that these subtypes are merely stages of disease rather than distinct entities. Elderly patients with MDS with trilineage dysplasia and greater than 30% myeloblasts who progress to acute leukemia are often considered to have a poor prognosis because their response rate to chemotherapy is lower than de novo acute myeloid leukemia patients. The World Health Organization (WHO) classification (1999) proposes to include all cases of RAEB-T, or patients with greater than 20% myeloblasts, in the category of acute leukemia because these patients have similar prognostic outcomes. However, their response to therapy is worse than the de novo or more typical acute myelogenous leukemia or acute non-lymphocytic leukemia (ANLL) patient. Id.

The fifth type of MDS, the most difficult to classify, is called chronic myelomonocytic leukemia (CMML). This subtype can have any percentage of myeloblasts but presents with a monocytosis of 1000/dL or more. It may be associated with splenomegaly. This subtype overlaps with a myeloproliferative disorder and may have an intermediate clinical course. It is differentiated from the classic chronic myelocytic leukemia (CML) that is characterized by a negative Ph chromosome. The recent WHO classification (1999) proposes that juvenile and proliferative CMML be listed separately from FAB under MDS/myeloproliferative disorder (MPD) with splenomegaly and greater than 13,000 total WBC. CMML is limited to monocytosis, less than 13,000/mm<sup>3</sup> total leukocytes, and requires trilineage dysplasia. Id. Harris N. L., et al., *J. Clin. Oncol.* 1999 December, 17(12): 3835-49. Finally, some other international organizations, including WHO, have suggested a sixth class of MDS patients, characterized by a del (5q) abnormality.

MDS is primarily a disease of elderly people, with the median onset in the seventh decade of life. The median age of these patients is 65 years, with ages ranging from the early third decade of life to as old as 80 years or older. The syndrome may occur in any age group, including the pediatric population. Patients who survive malignancy treatment with alkylating agents, with or without radiotherapy, have a high incidence of developing MDS or secondary acute leukemia.

About 60-70% of patients do not have an obvious exposure or cause for MDS, and are classified as primary MDS patients.

The most common cases of MDS are primary, or idiopathic. However, a nonspecific history of exposure to indeterminate chemicals or radiation 10-15 years prior to onset of disease may be present in about 50% of patients. This relationship to pathogenesis remains unproved. Compounds such as, but not limited to, benzene, insecticides, weed killers, and fungicides are possible causes of MDS. Goldberg H., et al., *Cancer Res.* 1990 Nov. 1; 50(21): 6876-81. Secondary MDS describes development of MDS or acute leukemia after known exposures to chemotherapy drugs that can cause bone marrow damage. These drugs are associated with a high incidence of chromosomal abnormalities following exposure and at the time of MDS or acute leukemia diagnosis.

Further, MDS is associated with complications associated with severe cytopenias. Other complications are development of myelofibrosis, which can accelerate decline in blood counts and increase transfusion requirements. Transformation to acute leukemia accelerates the development of complications such as anemia, bleeding, and infections.

The International MDS Risk Analysis (IMRA) Workshop proposed an International Prognosis Scoring System (IPSS) to decrease imprecision in predicting survival and AML risk in MDS patients. The IPSS is based on the number of cytopenias, percentage of BM blasts, and type of cytogenetic abnormalities (Table 1). Greenberg et al., *Blood* 1997, 89:2079-88. The latter are categorized into good (normal, —Y, del (5q), del (20q)), intermediate, and poor subgroups (complex or chromosome 7 abnormalities).

TABLE 1

International Prognostic Scoring System for MDS					
Prognostic Variable	Score Value				
	0	0.5	1.0	1.5	2.0
Bone marrow blasts (%)	<5	5-10	—	11-20	21-30
Karyotype*	Good	Intermediate	Poor		
Cytopenias	0/1	2/3			

\*Good, normal, del (5q), del (20q), -Y; Poor, complex (>3) or chromosome 7 abnormalities; Intermediate, +8, and other single or double abnormalities.

3.2. MDS Treatment

The current treatment of MDS is based on the stage and the mechanism of the disease that predominates the particular phase of the disease process. Bone marrow transplantation has been used in patients with poor prognosis or late-stage MDS. Epstein and Slease, 1985, *Surg. Ann.* 17:125. This type of therapy, however, is both painful for donor and recipient, because of the involvement of invasive procedures and can cause severe and even fatal complications to the recipient, particularly with allogeneic transplant and related Graft Versus Host Disease (GVHD) results. Therefore, the risk of GVHD restricts the use of bone marrow transplantation to patients with otherwise fatal diseases. Further, as most patients are elderly and only a few young MDS patients will have a matched donor, the use of bone marrow transplantation is limited.

An alternative approach to therapy for MDS is the use of hematopoietic growth factors or cytokines to stimulate blood cell development in a recipient. Dexter, 1987, *J. Cell Sci.* 88:1; Moore, 1991, *Annu. Rev. Immunol.* 9:159; and Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599-617. The process of blood cell formation, by which a small number of self-renewing stem cells give rise to lineage specific progeni-

tor cells that subsequently undergo proliferation and differentiation to produce the mature circulating blood cells has been shown to be at least in part regulated by specific hormones. These hormones are collectively known as hematopoietic growth factors. Metcalf, 1985, *Science* 229:16; Dexter, 1987, *J. Cell Sci.* 88:1; Golde and Gasson, 1988, *Scientific American*, July: 62; Tabbara and Robinson, 1991, *Anti-Cancer Res.* 11:81; Ogawa, 1989, *Environ. Health Persp.* 80:199; and Dexter, 1989, *Br. Med. Bull.* 45:337. The most well characterized growth factors include erythropoietin (EPO), granulocyte macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF). Apart from inducing proliferation and differentiation of hematopoietic progenitor cells, such cytokines have also been shown to activate a number of functions of mature blood cells, including influencing the migration of mature hematopoietic cells. Stanley et al., 1976, *J. Exp. Med.* 143:631; Schrader et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:323; Moore et al., 1980, *J. Immunol.* 125:1302; Kurland et al., 1979, *Proc. Natl. Acad. Sci. U.S.A.* 76:2326; Handman and Burgess, 1979, *J. Immunol.* 122:1134; Vadas et al., 1983, *Blood* 61:1232; Vadas et al., 1983, *J. Immunol.* 130:795; and Weibart et al., 1986, *J. Immunol.* 137:3584.

Unfortunately, hematopoietic growth factors have not proven effective in many clinical settings. Clinical trials of MDS patients treated with recombinant human GM-CSF and G-CSF have shown that while these cytokines can restore granulocytopenia in treated patients, their efficacy is restricted to the granulocyte or monocyte lineage with little or no improvement in hemoglobin or platelet counts. Schuster et al., 1990, *Blood* 76 (Suppl. 1):318a. When such patients were treated with recombinant human EPO, a sustained improvement in hemoglobin or decrease in transfusion requirement was achieved in only less than 25% of patients. Besa et al., 1990, 76 (Suppl. 1):133a; Hellstrom et al., 1990, 76 (Suppl. 1):279a; Bowen et al., 1991, *Br. J. Haematol.* 77:419. Therefore, there remains a need for safe and effective methods of treating and managing MDS.

3.3. Thalidomide and Azacitidine

Thalidomide is a racemic compound sold under the trade-name Thalomid® and chemically named  $\alpha$ -(N-phthalimido) glutarimide or 2-(2,6-dioxo-3-piperidinyl)-1H-isoindole-1,3 (2H)-dione. Thalidomide was originally developed in the 1950's to treat morning sickness, but due to its teratogenic effects was withdrawn from use. Thalidomide has been approved in the United States for the acute treatment of the cutaneous manifestations of erythema nodosum leprosum in leprosy. *Physicians' Desk Reference*, 1154-1158 (56<sup>th</sup> ed., 2002). Because its administration to pregnant women can cause birth defects, the sale of thalidomide is strictly controlled. Id. Thalidomide has reportedly been studied in the treatment of other diseases, such as chronic graft-vs-host disease, rheumatoid arthritis, sarcoidosis, several inflammatory skin diseases, and inflammatory bowel disease. See generally, Koch, H. P., *Prog. Med. Chem.* 22:165-242 (1985). See also, Moller, D. R., et al., *J. Immunol.* 159:5157-5161 (1997); Vasiliauskas, E. A., et al., *Gastroenterology* 117:1278-1287 (1999); Ehrenpreis, E. D., et al., *Gastroenterology* 117:1271-1277 (1999). It has further been alleged that thalidomide can be combined with other drugs to treat ischemia/reperfusion associated with coronary and cerebral occlusion. See U.S. Pat. No. 5,643,915, which is incorporated herein by reference.

More recently, thalidomide was found to exert immunomodulatory and anti-inflammatory effects in a variety of disease states, cachexia in AIDS, and opportunistic infections in AIDS. In studies to define the physiological targets of thali-

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domide, the drug was found to have a wide variety of biological activities exclusive of its sedative effect including neurotoxicity, teratogenicity, suppression of TNF- $\alpha$  production by monocytes/macrophages and the accompanying inflammatory toxicities associated with high levels of TNF- $\alpha$ , and inhibition of angiogenesis and neovascularization.

Additionally, beneficial effects have been observed in a variety of dermatological conditions, ulcerative colitis, Crohn's disease, Bechets's syndrome, systemic lupus erythematosus, aphthous ulcers, and lupus. The anti-angiogenic properties of thalidomide in in vivo models have been reported. D'Amato et al., *Thalidomide Is An Inhibitor Of Angiogenesis*, 1994, *PNAS, USA* 91:4082-4085.

One of the most therapeutically significant potential uses of thalidomide is in the treatment of cancer. The compound has been investigated in the treatment of various types of cancer, such as refractory multiple myeloma, brain, breast, colon, and prostate cancer, melanoma, mesothelioma, and renal cell carcinoma. See, e.g., Singhal, S., et al., *New England J. Med.* 341(21):1565-1571 (1999); and Marx, G. M., et al., *Proc. Am. Soc. Clin. Oncology* 18:454a (1999). Thalidomide reportedly can also be used to prevent the development of chronic cardiomyopathy in rats caused by doxorubicin. Costa, P. T., et al., *Blood* 92(10:suppl. 1):235b (1998). Other reports concerning the use of thalidomide in the treatment of specific cancers include its combination with carboplatin in the treatment of glioblastoma multiforme. McCann, J., *Drug Topics* 41-42 (Jun. 21, 1999). The use of thalidomide in combination with dexamethasone reportedly was effective in the treatment of patients suffering from multiple myeloma who also received, as supportive care, human granulocyte colony-stimulating factor (G-CSF), ciprofloxacin, and non-absorbable antifungal agents. Kropff, M. H., *Blood* 96(11 part 1):168a (2000); see also, Munshi, N. et al., *Blood* 94(10 part 1):578a (1999). Other chemotherapy combinations that comprise thalidomide are disclosed in International Application No. PCT/US01/15326 to R. Govindarajan and A. Zeitlan, and in International Application No. PCT/US01/15327 to J. B. Zeldis, et al.

In an effort to provide compounds that have greater therapeutic safety and efficacy than thalidomide, researchers have begun investigating a large number of other compounds, some of which are derivatives of thalidomide. See, e.g., Marriott, J. B., et al., *Expert Opin. Biol. Ther.* 1(4):1-8 (2001); G. W. Muller, et al., *Journal of Medicinal Chemistry* 39(17): 3238-3240 (1996); and G. W. Muller, et al., *Bioorganic & Medicinal Chemistry Letters* 8: 2669-2674 (1998). Examples include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles described in U.S. Pat. Nos. 6,281, 230 and 6,316,471, both to G. W. Muller, et al.

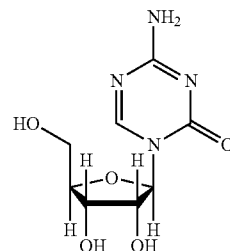
A group of compounds selected for their capacity to potently inhibit TNF- $\alpha$  production by LPS stimulated PBMC has been investigated. L. G. Corral, et al., *Ann. Rheum. Dis.* 58: (Suppl 1) 1107-1113 (1999). These compounds, which are referred to as IMiDs<sup>TM</sup> or Immunomodulatory Drugs, show not only potent inhibition of TNF- $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  and IL12 production. LPS induced IL6 is also inhibited by IMiDs<sup>TM</sup>, albeit partially. These compounds are potent stimulators of LPS induced IL10, increasing IL10 levels by 200 to 300%. Id.

In addition, nucleoside analogs have been used clinically for the treatment of viral infections and proliferative disorders for decades. Most of the nucleoside analog drugs are classified as antimetabolites. After they enter cells, nucleoside analogs are successively phosphorylated to nucleoside 5'-monophosphates, 5'-diphosphates, and 5'-triphosphates. In most

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cases, nucleoside triphosphates are the chemical entities that inhibit DNA or RNA synthesis, either through a competitive inhibition of polymerases or through incorporation of modified nucleotides into DNA or RNA sequences. Nucleosides may act also as their diphosphates.

5-Azacytidine (also known as azacitidine and 4-amino-1- $\beta$ -D-ribofuranosyl-1,3,5-triazin-2(1H)-one; Nation Service Center designation NSC-102816; CAS Registry Number 320-67-2) has undergone NCI-sponsored trials for the treatment of MDS. See, e.g., Kornblith et al., *J. Clin. Oncol.* 20(10): 2441-2452 (2002); Silverman et al., *J. Clin. Oncol.* 20(10): 2429-2440 (2002). 5-Azacytidine may be defined as having a molecular formula of C<sub>8</sub>H<sub>12</sub>N<sub>4</sub>O<sub>5</sub>, a relative molecular weight of 244.21 and a structure of:



Azacytidine (also referred to as 5-azacytidine herein) is a nucleoside analog, more specifically a cytidine analog. 5-Azacytidine is an antagonist of its related natural nucleoside, cytidine. 5-Azacytidine, as well as decitabine, i.e., 5-aza-2'-deoxycytidine, are antagonists of decitabine's related natural nucleoside, deoxycytidine. The only structural difference between the analogs and their related natural nucleosides is the presence of nitrogen at position 5 of the cytosine ring in place of oxygen.

Other members of the class of deoxycytidine and cytidine analogs include arabinosylcytosine (Cytarabine), 2'-deoxy-2',2'-difluorocytidine (Gemcitabine), 5-aza-2'-deoxycytidine (Decitabine), 2(1H)-pyrimidine-ribose (Zebularine), 2',3'-dideoxy-5-fluoro-3'-thiacytidine (Emtriva), N<sup>4</sup>-pentylloxycarbonyl-5'-deoxy-5-fluorocytidine (Capecitabine), 2'-cyclocytidine, arabinofuranosyl-5-azacytidine, dihydro-5-azacytidine, N<sup>4</sup>-octadecyl-cytarabine, elaidic acid cytarabine, and cytosine 1- $\beta$ -D-arabinofuranoside (ara-C).

There remains a need for more effective methods for treating MDS and its related disorders.

## 4. SUMMARY

Embodiments herein provide methods for the treatment of myelodysplastic syndromes (MDS) with a combination therapy using a therapeutically effective amount of a cytidine analog, including, but not limited to, 5-azacytidine, and a therapeutically effective amount of an immunomodulatory compound, including, but not limited to, 3-(4-amino-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (lenalidomide) or a pharmaceutically acceptable salt, solvate (e.g., hydrate) or stereoisomer thereof. Particular embodiments provide methods for treating patients with advanced MDS or higher risk MDS using the combination therapy. Particular embodiments provide methods for improving the overall survival of patients having MDS, e.g., higher risk MDS. Particular embodiments provide alternative dosing regimens for treating MDS. Particular embodiments provide methods for treating patients having MDS, using specific numbers of lenalidomide and azacitidine treatment cycles. Particular embodi-



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ments provide methods of treating patients who meet the WHO criteria for AML using lenalidomide and azacitidine. Particular embodiments provide using lenalidomide and azacitidine as maintenance therapy.

One embodiment encompasses the use of lenalidomide and azacitidine in combination with conventional therapies presently used to treat, prevent or manage MDS such as hematopoietic growth factors, cytokines, cancer chemotherapeutics, stem cell transplantation and other transplantations.

## 5. DETAILED DESCRIPTION

A first embodiment encompasses methods of treating or preventing MDS which comprise administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of 3-(4-amino-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (lenalidomide), or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with a therapeutically or prophylactically effective amount of a cytidine analog, particularly, 5-azacytidine. The embodiment encompasses the treatment, prevention or management of specific sub-types of MDS such as refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia.

As used herein, the term “myelodysplastic syndromes” or “MDS” means hematopoietic stem cell disorders characterized by one or more of the following: ineffective blood cell production, progressive cytopenias, risk of progression to acute leukemia or cellular marrow with impaired morphology and maturation (dysmyelopoiesis). The term “myelodysplastic syndromes” or “MDS” unless otherwise noted includes: refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia.

Another embodiment encompasses methods of managing MDS which comprises administering to a patient in need of such management a prophylactically effective amount of lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with a therapeutically or prophylactically effective amount of 5-azacytidine.

Another embodiment encompasses a kit comprising: a pharmaceutical composition comprising (1) lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and (2) 5-azacytidine, and/or (3) instructions for use. The invention further encompasses kits comprising single unit dosage forms.

One embodiment encompasses a method of treating, preventing and/or managing MDS, which comprises administering to a patient in need of such treatment, prevention and/or management therapeutically or prophylactically effective amounts of 5-azacytidine and lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a therapeutically or prophylactically effective amount of an additional active agent.

The additional active agent is preferably a hematopoietic growth factor, a cytokine, an anti-cancer agent, an antibiotic, an anti-fungal, an anti-inflammatory, an immunosuppressive agent such as a cyclosporin, conventional therapy for MDS, or other chemotherapeutic agent found for example in the Physician's Desk Reference. Preferred anti-cancer or cancer chemotherapeutics are apoptosis inducing agents, topoisomerase inhibitors, anti-angiogenesis compounds, microtubule stabilizing agents, alkylating agents and other known

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conventional cancer chemotherapy. Most preferred additional active agents are those capable of affecting or improving blood production. The active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules). The examples of specific additional active agent include, but are not limited to, gemtuzamab, ozogamicin, etanercept (Enbrel®), imatinib (Gleevec®), anti-TNF- $\alpha$  antibodies, infliximab (Remicade®), G-CSF, GM-CSF, EPO, topotecan, irinotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, vinblastine, isotretinoin, and 13-cis-retinoic acid. This invention also encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term “mutants” are proteins that lack carbohydrate moieties normally present in their naturally occurring forms (e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001). Vaccines that cause the secretion of proteins disclosed herein as well as pharmacologically active mutants, derivatives, and fusion thereof are also encompassed by the invention.

Without being limited by theory, it is believed that certain immunomodulatory compounds and proteins can act in complementary or synergistic ways in the treatment or management of MDS. It is also believed that certain proteins may reduce or eliminate particular adverse effects associated with some immunomodulatory compounds, thereby allowing the administration of larger amounts of an immunomodulatory compound to patients and/or increasing patient compliance. It is further believed that some immunomodulatory compounds may reduce or eliminate particular adverse effects associated with some protein-based MDS therapies, thereby allowing the administration of larger amounts of protein to patients and/or increasing patient compliance.

Another embodiment of the invention encompasses a method of reversing, reducing or avoiding an adverse effect associated with the administration of a chemotherapeutics or therapeutics used to treat cancer or MDS in a patient suffering from MDS, which comprises administering to a patient in need thereof therapeutically or prophylactically effective amounts of azacitidine and lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

As inevitable leukemic transformation develops in certain stages of MDS, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. It is believed that the combined use of an immunomodulatory compound and transplantation of stem cells in a patient suffering from MDS provides a unique and unexpected synergism. In particular, without being limited by theory, it is believed that an immunomodulatory compound exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy. Immunomodulatory compounds can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of related Graft Versus Host Disease

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(GVHD). Therefore, this invention encompasses a method of treating, preventing and/or managing MDS, which comprises administering to a patient (e.g., a human) azacitidine and lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after transplantation therapy.

The invention also encompasses pharmaceutical compositions, single unit dosage forms, and kits which comprise azacitidine and lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, an additional active ingredient, and/or blood or cells for transplantation therapy. For example, the kit may contain one or more compounds of the invention, stem cells for transplantation and an immunosuppressive agent, antibiotic or other drug, each of which is to be used to treat the MDS patient.

### 5.1. Immunomodulatory Compounds

Compounds used in the invention include immunomodulatory compounds that are racemic, stereomerically enriched or stereomerically pure, and pharmaceutically acceptable salts, solvates, hydrates, stereoisomers, clathrates, and prodrugs thereof. Preferred compounds used in the invention are small organic molecules having a molecular weight less than about 1000 g/mol, and are not proteins, peptides, oligonucleotides, oligosaccharides or other macromolecules.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. As used herein and unless otherwise indicated, the term "stereomerically enriched" means a composition that comprises greater than about 60% by weight of one stereoisomer of a compound, preferably greater than about 70% by weight, more preferably greater than about 80% by weight of one stereoisomer of a compound. As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center. Similarly, the term "stereomerically enriched" means a stereomerically enriched composition of a compound having one chiral center.

As used herein and unless otherwise indicated, the term "immunomodulatory compounds" or "ImiDs™" (Celgene Corporation) used herein encompasses small organic molecules that markedly inhibit TNF- $\alpha$ , LPS induced monocyte IL1 $\beta$  and IL12, and partially inhibit IL6 production. Specific immunomodulatory compounds of the invention are discussed below.

TNF- $\alpha$  is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation. TNF- $\alpha$  is responsible for a diverse range of signaling events within

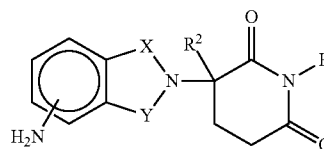
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cells. TNF- $\alpha$  may play a pathological role in cancer. Without being limited by particular theory, one of the biological effects exerted by the immunomodulatory compounds of the invention is the reduction of synthesis of TNF- $\alpha$ . Immunomodulatory compounds of the invention enhance the degradation of TNF- $\alpha$  mRNA.

Further, without being limited by particular theory, immunomodulatory compounds used in the invention may also be potent co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. Immunomodulatory compounds of the invention may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset. In addition, the compounds preferably have anti-inflammatory properties, and efficiently co-stimulate T cells.

Specific examples of immunomodulatory compounds of the invention, include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl)isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl) isoindolines such as those described in U.S. Pat. No. 5,874,448; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolines described in U.S. Pat. No. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines (e.g., 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517; and a class of non-polypeptide cyclic amides disclosed in U.S. Pat. Nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S. Pat. Nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles such as those described in U.S. Pat. Nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those described in U.S. patent application Ser. No. 09/972,487 filed on Oct. 5, 2001, U.S. patent application Ser. No. 10/032,286 filed on Dec. 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents identified herein are incorporated herein by reference. Immunomodulatory compounds of the invention do not include thalidomide.

Other specific immunomodulatory compounds of the invention include, but are not limited to, 1-oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein. These compounds have the structure I:



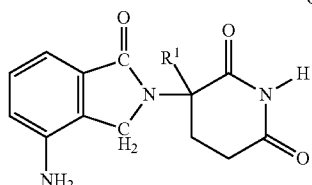
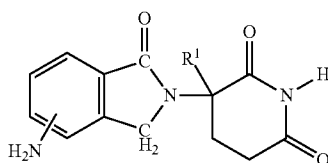
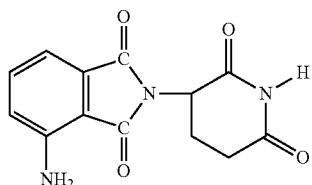
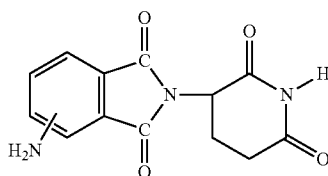
in which one of X and Y is C=O, the other of X and Y is C=O or CH<sub>2</sub>, and R<sup>2</sup> is hydrogen or lower alkyl, in particular methyl. Specific immunomodulatory compounds include, but are not limited to:

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1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
 1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;  
 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;  
 1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;  
 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
 and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

Other specific immunomodulatory compounds of the invention belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles, such as those described in U.S. Pat. Nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (International Publication No. WO 98/03502), each of which is incorporated herein. Compounds representative of this class are of the formulas:

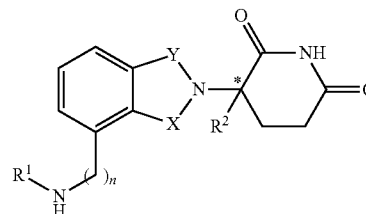


wherein  $R^1$  is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (e.g. optically pure (R) or (S) enantiomers) of these compounds.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. Nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are incorporated herein by reference. Representative compounds are of formula II:

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II



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

$R^1$  is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, C(O)R<sup>3</sup>, C(S)R<sup>3</sup>, C(O)OR<sup>4</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, C(O)NHR<sup>3</sup>, C(S)NHR<sup>3</sup>, C(O)NR<sup>3</sup>R<sup>3'</sup>, C(S)NR<sup>3</sup>R<sup>3'</sup> or (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>;

$R^2$  is H, F, benzyl, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, or (C<sub>2</sub>-C<sub>8</sub>)alkynyl;

$R^3$  and  $R^{3'}$  are independently (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>0</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>, or C(O)OR<sup>5</sup>;

$R^4$  is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, (C<sub>1</sub>-C<sub>4</sub>)alkyl-OR<sup>5</sup>, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, or (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl;

$R^5$  is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, or (C<sub>2</sub>-C<sub>5</sub>)heteroaryl;

each occurrence of  $R^6$  is independently H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>2</sub>-C<sub>5</sub>)heteroaryl, or (C<sub>0</sub>-C<sub>8</sub>)alkyl-C(O)O—R<sup>5</sup> or the  $R^6$  groups can join to form a heterocycloalkyl group;

n is 0 or 1; and

\* represents a chiral-carbon center.

In specific compounds of formula II, when n is 0 then  $R^1$  is (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, C(O)R<sup>3</sup>, C(O)OR<sup>4</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, C(S)NHR<sup>3</sup>, or (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>;

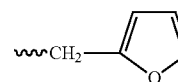
$R^2$  is H or (C<sub>1</sub>-C<sub>8</sub>)alkyl; and

$R^3$  is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>5</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>; (C<sub>0</sub>-C<sub>8</sub>)alkyl-NH—C(O)O—R<sup>5</sup>; (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>, or C(O)OR<sup>5</sup>; and the other variables have the same definitions.

In other specific compounds of formula II,  $R^2$  is H or (C<sub>1</sub>-C<sub>4</sub>)alkyl.

In other specific compounds of formula II,  $R^1$  is (C<sub>1</sub>-C<sub>8</sub>)alkyl or benzyl.

In other specific compounds of formula II,  $R^1$  is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, benzyl, CH<sub>2</sub>OCH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, or

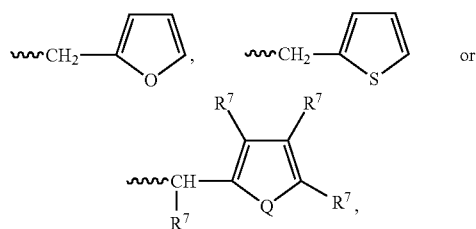




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In another embodiment of the compounds of formula II, R<sup>1</sup> is



wherein Q is O or S, and each occurrence of R<sup>7</sup> is indepen- 15  
dently H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, benzyl, CH<sub>2</sub>OCH<sub>3</sub>, or  
CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>.

In other specific compounds of formula II,  $R^1$  is  $C(O)R^3$ .

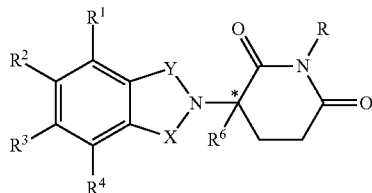
In other specific compounds of formula II, R<sup>3</sup> is (C<sub>0</sub>-C<sub>4</sub>) alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>1</sub>-C<sub>8</sub>)alkyl, aryl, or (C<sub>0</sub>-C<sub>4</sub>)alkyl-OR<sup>5</sup>.

In other specific compounds of formula II, heteroaryl is pyridyl, furyl, or thieryl.

In other specific compounds of formula II, R<sup>1</sup> is C(O)OR<sup>4</sup>.

In other specific compounds of formula II, the H of C(O)NHC(O) can be replaced with (C<sub>1</sub>-C<sub>4</sub>)alkyl, aryl, or benzyl.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. No. 09/781,179, International Publication No. WO 98/54170, and U.S. Pat. No. 6,395,754, each of which are incorporated herein by reference. Representative compounds are of formula III:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

R is H or  $\text{CH}_2\text{OCOR}'$ ;

(i) each of  $R^1, R^2, R^3$ , or  $R^4$ , independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of  $R^1, R^2, R^3$ , or  $R^4$  is nitro or  $-NHR^5$  and the remaining of  $R^1, R^2, R^3$ , or  $R^4$  are hydrogen;

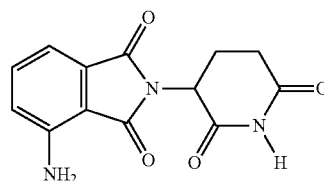
R<sup>5</sup> is hydrogen or alkyl of 1 to 8 carbons;

R<sup>6</sup> hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro; and

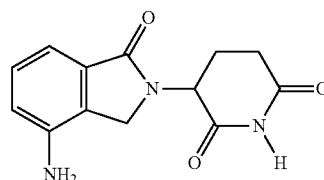
\* represents a chiral-carbon center.

The most preferred immunomodulatory compounds of the invention are 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. The compounds can be obtained via standard, synthetic methods (see e.g., U.S. Pat. No. 5,635, 517, incorporated herein by reference). The compounds are available from Celgene Corporation, Summit, N.J. 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (pomalidomide) has the following chemical structure:

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3-(4 Amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (lenalidomide) has the following chemical structure:



The compounds of the invention can either be commercially purchased or prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry techniques.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable salt" encompasses non-toxic acid and base addition salts of the compound to which the term refers. Acceptable non-toxic acid addition salts include those derived from organic and inorganic acids or bases known in the art, which include, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methane-sulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embolic acid, enanthic acid, and the like.

Compounds that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds are those that form non-toxic base addition salts, i.e., salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), lysine, and procaine.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of immunomodulatory compounds of the invention that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of immunomodulatory compounds of the invention that comprise —NO, —NO<sub>2</sub>, —ONO, or —ONO<sub>2</sub> moieties. Prodrugs can typically be prepared using well known methods, such as those described in

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1 *Burger's Medicinal Chemistry and Drug Discovery*, 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995), and *Design of Prodrugs* (H. Bundgaard ed., Elsevier, New York 1985).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxymethyl, acetoxylethyl, aminocarbonyloxymethyl, pivaloyloxymethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyloxymethyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides,  $\alpha$ -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

#### 5.2. Additional Active Agents

One or more additional active ingredients can be used in the methods and compositions of the invention together with azacitidine and an immunomodulatory compound of the invention. In a preferred embodiment, the additional active agents are capable of affecting or improving the process of blood cell production. Specific additional active agents also stimulate the division and differentiation of committed erythroid progenitors in cells in vitro or in vivo.

Additional active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules). The additional active agents include but are not limited to hematopoietic growth factors, cytokines, anti-cancer agents, antibiotics, proteasome inhibitors, immunosuppressive agents and other therapeutics discussed herein. Particular agents include, but are not limited to, G-CSF, GM-CSF, EPO, dexamethasone, topotecan, pentoxifylline, irinotecan, ciprofloxacin, vinorelbine, IL2, IL8, IL18, Ara-C, isotretinoin, 13-cis-retinoic acid, 12-O-tetradecanoylphorbol-13-acetate (TPA), 5-AZA2'-deoxycytidine, 9-nitrocamp-tothecin, transretinoic acid, amifostine, amphotericin B and liposomal amphotericin B, anti-CD-20 monoclonal antibody, anti-thymocyte globulin (ATG), arsenic trioxide, azacitidine, bevacizumab, bismuth monoclonal antibody, bryostatins, busulfan, caspofungin acetate, celastrol, cladribine, cyclophosphamide, cyclosporine, cytarabine, cytosine, daunorubicin, decapeptide, etoposide, farfesy transferase inhibitor, flavopiridol, Flt3 ligand, fludarabine, gentuzumab ozogomicin (mylotarg), etanercept (Enbrel®), imatinib (Glivec®), anti-TNF- $\alpha$  antibodies, infliximab

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(Remicade®), humanized monoclonal anti-VEGF antibody, idarubicin, leucovorin, melphalan, mitoxantrone, monoclonal antibody ABX-CBL, monoclonal antibody CD52, mycophenolate mofetil, oblimersen, omega-3 fatty acids, pentostatin, phenylbutyrate, PR1 leukemia peptide vaccine, montanide, proteasome inhibitor, sodium phenylbutyrate, sodium salicylate, temozolomide, thymoglobulin, troxatyl, tumor necrosis factor receptor IgG chimera, Yttrium Y 90 humanized monoclonal antibody M195. In a specific embodiment of the invention, an immunomodulatory compound of the invention is used in combination with pentoxifylline, ciprofloxacin, and/or dexamethasone.

This invention also encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term "mutants" are proteins that lack carbohydrate moieties normally present in their naturally occurring forms (e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001).

Recombinant and mutated forms of G-CSF can be prepared as described in U.S. Pat. Nos. 4,810,643; 4,999,291; 5,528,823; and 5,580,755; all of which are incorporated herein by reference. Recombinant and mutated forms of GM-CSF can be prepared as described in U.S. Pat. Nos. 5,391,485; 5,393,870; and 5,229,496; all of which are incorporated herein by reference. In fact, recombinant forms of G-CSF and GM-CSF are currently sold in the United States for the treatment of symptoms associated with specific chemotherapies. A recombinant form of G-CSF known as filgrastim is sold in the United States under the trade name NEUPOGEN®. NEUPOGEN® is known to stimulate division and maturation of granulocytes, mostly neutrophils, in MDS patients and to enhance erythroid response in combination with EPO. *Physicians' Desk Reference*, 587-592 (56<sup>th</sup> ed., 2002). A recombinant form of GM-CSF known as sargramostim is also sold in the United States under the trade name LEUKINE®. LEUKINE® is known to stimulate division and maturation of earlier myeloid and macrophage precursor cells and has been reported to increase granulocytes. *Physicians' Desk Reference*, 1755-1760 (56<sup>th</sup> ed., 2002). A recombinant form of EPO known as epoetin alfa is sold in the United States under the trade name EPOGEN®. EPOGEN® is used to stimulate red cell production by stimulating division and maturation of committed red cell precursor cells. EPOGEN® has been reported to be effective in 20-26% of MDS patient when administered by itself and in as many as 48% of patients when combined with G-CSF or GM-CSF. *Physicians' Desk Reference*, 582-587 (56<sup>th</sup> ed., 2002).

A growth-factor or cytokine such as G-CSF, GM-CSF and EPO can also be administered in the form of a vaccine. For example, vaccines that secrete, or cause the secretion of, cytokines such as G-CSF and GM-CSF can be used in the methods, pharmaceutical compositions, and kits of the invention. See, e.g., Emens, L. A., et al., *Curr. Opinion Mol. Ther.* 3(1):77-84 (2001).

Other compounds that can be administered or used in combination with an immunomodulatory compound of the inven-

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tion include those disclosed in U.S. provisional patent application No. 60/380,842, filed May 17, 2002, and U.S. provisional patent application No. 60/380,843, filed May 17, 2002, both of which are incorporated herein by reference.

### 5.3. Methods of Treatment and Management

Methods of this invention encompass methods of preventing, treating and/or managing various types of MDS. As used herein, unless otherwise specified, the term “preventing” includes but is not limited to, inhibition or the averting of symptoms associated with MDS. The symptoms associated with MDS include, but are not limited to, anemia, thrombocytopenia, neutropenia, cytopenia, bicytopenia (two deficient cell lines), and pancytopenia (three deficient cell lines). As used herein, unless otherwise specified, the term “treating” refers to the administration of a composition after the onset of symptoms of MDS, whereas “preventing” refers to the administration prior to the onset of symptoms, particularly to patients at risk of MDS. As used herein and unless otherwise indicated, the term “managing” encompasses preventing the recurrence of MDS in a patient who had suffered from MDS, lengthening the time a patient who had suffered from MDS remains in remission, and/or preventing the occurrence of MDS in patients at risk of suffering from MDS.

The invention encompasses methods of treating or preventing patients with primary and secondary MDS. It further encompasses methods treating patients who have been previously treated for MDS, as well as those who have not previously been treated for MDS. Because patients with MDS have heterogenous clinical manifestations and varying clinical outcomes, it has become apparent that staging the patients according to their prognosis and approaching therapy depending on the severity and stage is necessary. Indeed, the methods and compositions of this invention can be used in various stages of treatments for patients with one or more types of MDS including, but not limited to, refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess blasts (RAEB), RAEB in transformation (RAEB-T), or chronic myelomonocytic leukemia (CMML). The invention also contemplates treating patients diagnosed using the IPSS for MDS discussed above. Greenberg et al., *Blood* 1997 (89): 2079-88.

Methods encompassed by this invention comprise administering azacitidine and an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof to a patient (e.g., a human) suffering, or likely to suffer, from MDS. Specific patient populations include the elderly, i.e., ages 60 and above as well as those over 35 years of age. Patients with familial history of MDS or leukemia are also preferred candidates for preventive regimens.

In one embodiment of the invention, an immunomodulatory compound of the invention is administered orally and in a single or divided daily doses in an amount of from about 0.10 to about 150 mg/day. In a particular embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (pomalidomide) is administered in an amount of from about 0.1 to about 10 mg per day, or alternatively every other day. 3-(4-Amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (lenalidomide) can be preferably administered in an amount of from about 5 to 25 mg per day, or alternatively every other day.

In one embodiment of the invention, azacitidine is administered according to one of three alternative dosing schedules, administered in 28-day cycles:

- 1) AZA 5-2-2: azacitidine 75 mg/m<sup>2</sup>/day SC×5 days, followed by 2 days of no treatment, followed by azacitidine 75 mg/m<sup>2</sup>/day SC×2 days;

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- 2) AZA 5-2-5: azacitidine 50 mg/m<sup>2</sup>/day SC×5 days, followed by 2 days of no treatment, followed by azacitidine 50 mg/m<sup>2</sup>/day SC×5 days; and

- 3) AZA 5: azacitidine 75 mg/m<sup>2</sup>/day SC×5 days.

After at least 2 cycles, azacitidine dose can be increased if the patient is not responding, defined as treatment failure or disease progression according to IWG 2000 criteria for MDS ( $\geq 50\%$  increase in blasts,  $\geq 50\%$  decrease from maximum response levels in granulocytes or platelets, hemoglobin reduction  $\geq 2$  g/dL, or transfusion independence). Conversely, the dose can be decreased based on hematological recovery and adverse events.

### 5.3.1 Combination Therapy with Additional Active Agent

Particular methods of the invention comprise administering 1) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, 2) azacitidine and 3) an additional active agent or active ingredient. Examples of immunomodulatory compounds of the invention are disclosed herein (see, e.g., section 5.1); and examples of the additional active agents are also disclosed herein (see, e.g., section 5.2).

Administration of the immunomodulatory compounds, azacitidine and the additional active agents to a patient can occur simultaneously or sequentially by the same or different routes of administration. The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. A preferred route of administration for an immunomodulatory compound is oral. A preferred route of administration for azacitidine is subcutaneous, intravenous or oral. Preferred routes of administration for the additional active agents or ingredients of the invention are known to those of ordinary skill in the art. See, e.g., *Physicians' Desk Reference*.

In one embodiment, the additional active agent is administered intravenously or subcutaneously and once or twice daily in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. The specific amount of the additional active agent will depend on the specific agent used, the type of MDS being treated or managed, the severity and stage of MDS, and the amount(s) of immunomodulatory compounds of the invention and any optional additional active agents concurrently administered to the patient. In a particular embodiment, the additional active agent is gemtuzumab, ozogamicin, etanercept, GM-CSF, G-CSF, EPO, transretinoic acid, dexamethasone, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, or a combination thereof. GM-CSF is administered in an amount of from about 60 to about 500 mcg/m<sup>2</sup> intravenously over 2 hours, or from about 5 to about 12 mcg/m<sup>2</sup>/day subcutaneously. G-CSF is administered subcutaneously in an amount of about 1 mcg/kg/day initially and can be adjusted depending on rise of total granulocyte counts. The maintenance dose is 300 (in smaller patients) or 480 mcg subcutaneously. EPO is administered subcutaneously in an amount of 10,000 Unit 3 times per week.

### 5.3.2 Use with Transplantation Therapy

In still another embodiment, this invention encompasses a method of treating, preventing and/or managing MDS, which comprises administering azacitidine and the immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with transplantation therapy.



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As discussed elsewhere herein, the treatment of MDS is based on the stages and mechanism of the disease. As inevitable leukemic transformation develops in certain stages of MDS, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. The combined use of azacitidine, the immunomodulatory compound of the invention and transplantation therapy provides a unique and unexpected synergism. In particular, an immunomodulatory compound of the invention exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy in patients with MDS. An immunomodulatory compound of the invention can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of related Graft Versus Host Disease (GVHD). This invention encompasses a method of treating, preventing and/or managing MDS which comprises administering to a patient (e.g., a human) azacitidine and an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after the transplantation of umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow. Examples of stem cells suitable for use in the methods of the invention are disclosed in U.S. provisional patent application No. 60/372,348, filed Apr. 12, 2002 by R. Hariri et al., the entirety of which is incorporated herein by reference.

### 5.3.3 Cycling Therapy

In a preferred embodiment, the therapeutic agents of the invention are cyclically administered to a patient. Cycling therapy involves the administrations of the first agent and the second agent for periods of time, followed by rest for periods of time, and repeating the sequential administrations. Cycling therapy can reduce the development of resistance to the therapies, avoid or reduce the side effects of the therapies, and/or improves the efficacy of the treatment.

In a particular embodiment, one cycle can comprise the administration of a therapeutic agent for three (3) weeks followed by one (1) week of rest in a 28-days cycle. The number of cycles administered is from about 1 to about 12 cycles. 3-(4-Amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidin-2,6-dione (lenalidomide) is orally administered to patients with MDS in an amount of about 5-25 mg per day for 21 days followed by seven days rest on a 28 day cycle. In a particular embodiment, lenalidomide is orally administered to patients with MDS in an amount of about 10 mg per day for 21 days followed by seven days rest on a 28 day cycle.

In a particular embodiment, the patient also receive 5-azacytidine at a dose of about 25-75 mg/m<sup>2</sup>/d subcutaneously (SC) or intravenously (IV) for days 1-7 followed by twenty-one days rest on a 28 day cycle. In another particular embodiment, patient receive 5-azacytidine at a dose of about 25-75 mg/m<sup>2</sup>/d subcutaneously (SC) or intravenously (IV) for days 1-5 followed by twenty-three days rest on a 28 day cycle. In another particular embodiment, patient receive oral 5-azacytidine at a dose of about 120 mg/d for days 1-7 followed by twenty-one days rest on a 28 day cycle.

In one embodiment, up to 9 or more 28-day cycles are administered. Other methods for providing an effective amount of a cytidine analog are disclosed in, for example, "Colon-Targeted Oral Formulations of Cytidine Analogs", U.S. Ser. No. 11/849,958, which is incorporated by reference herein in its entirety.

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### 5.4. Pharmaceutical Compositions and Single Unit Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Pharmaceutical compositions and dosage forms of the invention can further comprise one or more excipients.

Pharmaceutical compositions and dosage forms of the invention can also comprise one or more additional active ingredients. Consequently, pharmaceutical compositions and dosage forms of the invention comprise the active ingredients disclosed herein (e.g., an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and an additional active ingredient). Examples of optional additional active ingredients are disclosed herein (see, e.g., section 5.2).

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), or parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), transdermal or transcutaneous administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18<sup>th</sup> ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients may be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage

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forms that contain little, if any, lactose other mono- or disaccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the *U.S. Pharmacopeia* (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of from about 0.10 to about 150 mg. Typical dosage forms comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of about 0.1, 1, 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 50, 100, 150 or 200 mg. In a particular embodiment, a preferred dosage form comprises 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione

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(Actimid™) in an amount of about 1, 2, 5, 10, 25 or 50 mg. In a specific embodiment, a preferred dosage form comprises 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine 2,6-dione (Lenalidomide) in an amount of about 5, 10, 25 or 50 mg.

Pharmaceutical compositions may contain sufficient quantities of azacitidine to provide a daily dosage of about 10 to 150 mg/m<sup>2</sup> (based on patient body surface area) or about 0.1 to 4 mg/kg (based on patient body weight) as single or divided (2-3) daily doses. In one embodiment, dosage of azacitidine is provided via a seven day administration of 75 mg/m<sup>2</sup> subcutaneously, once every twenty-eight days, for as long as clinically necessary.

Typical dosage forms comprise the additional active ingredient in an amount of 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. Of course, the specific amount of the additional active ingredient will depend on the specific agent used, the type of MDS being treated or managed, and the amount(s) of immunomodulatory compounds and azacitidine, and any optional additional active agents concurrently administered to the patient.

#### 5.4.1 Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences*, 18<sup>th</sup> ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other

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starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

A preferred solid oral dosage form of the invention comprises an immunomodulatory compound of the invention,

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anhydrous lactose, microcrystalline cellulose, polyvinylpyrrolidone, stearic acid, colloidal anhydrous silica, and gelatin.

#### 5.4.2 Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydroxypropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

#### 5.4.3 Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose



Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention. For example, cyclodextrin and its derivatives can be used to increase the solubility of an immunomodulatory compound of the invention, and its derivatives. See, e.g., U.S. Pat. No. 5,134,127, which is incorporated herein by reference.

5.4.4 Topical And Mucosal Dosage Forms

Topical and mucosal dosage forms of the invention include, but are not limited to, sprays, aerosols, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide topical and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form solutions, emulsions or gels, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990).

The pH of a pharmaceutical composition or dosage form may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

5.4.5 Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a dosage form of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, pro-drug, or clathrate thereof. Kits encompassed by this invention can further comprise additional active ingredients such as G-CSF, GM-CSF, EPO, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13-cis-retinoic acid, or a pharmacologically active mutant or derivative thereof, or a combination thereof.

Examples of the additional active ingredients include, but are not limited to, those disclosed herein (see, e.g., section 5.2).

Kits of the invention can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

6. EXAMPLES

The following studies are intended to further illustrate the invention without limiting its scope.

6.1. Clinical Studies in MDS Patients

Study 1

A Phase I trial was conducted in patients with MDS (IPSS score  $\geq 1.5$ , or FAB or WHO classification with  $\geq 5\%$  myeloblasts). Patients were treated using below dosing schedules.

Dose Level	Azacitidine Schedule	Lenalidomide Schedule
1	75 mg/m <sup>2</sup> SC days 1-5	5 mg PO days 1-14
2	75 mg/m <sup>2</sup> SC days 1-5	5 mg PO days 1-21
3	75 mg/m <sup>2</sup> SC days 1-5	10 mg PO days 1-21
4	50 mg/m <sup>2</sup> SC days 1-5, 8-12	5 mg PO days 1-14
5	50 mg/m <sup>2</sup> SC days 1-5, 8-12	5 mg PO days 1-21
6	50 mg/m <sup>2</sup> SC days 1-5, 8-12	10 mg PO days 1-21

Lenalidomide and Azacitidine were cyclically administered to patients with MDS. Cycling therapy involved the administration of the agents for a period of time, followed by rest for a period of time, and repeating this sequential administration. Cycles lasted 28 days, to a maximum of 7 cycles of therapy. The primary endpoint was to determine the maximum tolerated dose and dose-limiting toxicities of the combination. The secondary endpoint was response as defined by the Modified International Working Group (IWG). The combination of lenalidomide and Azacitidine was well-tolerated and the results showed efficacy in MDS patients.

Study 2

Patients receive treatment with lenalidomide at a oral dose of 5-25 mg daily on days 1 through 21, and Azacitidine at a dose of 25-75 mg/m<sup>2</sup>/d subcutaneously (SC) or intravenously (IV) on days 1 through 7 in cycles of 28 days. Patients receive treatments until disease progression or unacceptable toxicity.



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The combination of lenalidomide and Azacitidine is well tolerated with encouraging clinical activity. The results of this study indicate that the combination of lenalidomide and Azacitidine provides a highly effective treatment for MDS. These agents may complement each other by targeting both the bone marrow microenvironment and hypomethylating action on the malignant clone.

## Study 3

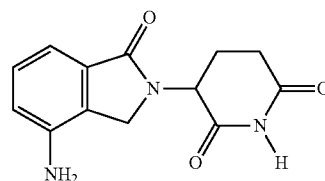
Patients receive continuous treatment with lenalidomide at a oral dose of 5-25 mg daily on days 1 through 21, and Azacitidine at a oral dose of 120 mg per day on days 1 through 7 in every 28 days cycle. Patients receive treatments until disease progression or unacceptable toxicity. The results indicate that the combination dosing regimen of lenalidomide and oral Azacitidine is active and well tolerated, with a manageable side effect profile. This therapy provides a highly effective treatment for MDS.

Embodiments of the invention described herein are only a sampling of the scope of the invention. The full scope of the invention is better understood with reference to the attached claims.

## What is claimed is:

1. A method of treating a patient having transfusion dependent anemia due to low to intermediate-1-risk myelodysplastic syndrome, which comprises administering to said patient in need thereof about 5 to about 25 mg per day of 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione having the formula:

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or a pharmaceutically acceptable salt, solvate or stereoisomer thereof.

2. The method of claim 1, wherein the compound is administered in the amount of 5 mg per day.

3. The method of claim 2, wherein the compound is administered orally in an amount of 5 mg as a capsule per day.

4. The method of claim 1, wherein the compound is administered in the amount of 10 mg per day.

5. The method of claim 4, wherein the compound is administered orally in an amount of 10 mg as a capsule per day.

6. The method of claim 1, wherein the compound is administered in the amount of 15 mg per day.

7. The method of claim 6, wherein the compound is administered orally in an amount of 15 mg as a capsule per day.

8. The method of claim 1, wherein the compound is administered in the amount of 25 mg per day.

9. The method of claim 8, wherein the compound is administered orally in an amount of 25 mg as a capsule per day.

10. The method of claim 3, 5, 7 or 9, wherein the compound 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione is as a free base.

\* \* \* \* \*

# **EXHIBIT F**



US008530498B1

(12) **United States Patent**  
**Zeldis**

(10) **Patent No.:** **US 8,530,498 B1**  
(45) **Date of Patent:** **\*Sep. 10, 2013**

(54) **METHODS FOR TREATING MULTIPLE MYELOMA WITH 3-(4-AMINO-1-OXO-1,3-DIHYDROISOINDOL-2-YL)PIPERIDINE-2,6-DIONE**

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(22) Filed: **Apr. 8, 2013**

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(63) Continuation of application No. 13/488,888, filed on Jun. 5, 2012, which is a continuation of application No. 12/640,702, filed on Dec. 17, 2009, now Pat. No. 8,198,306, which is a continuation of application No. 10/438,213, filed on May 15, 2003, now Pat. No. 7,968,569.

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**A01N 43/40** (2006.01)  
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(52) **U.S. Cl.**  
USPC ..... **514/320; 514/323**

(58) **Field of Classification Search**  
USPC ..... **514/323**  
See application file for complete search history.

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(57) **ABSTRACT**

Methods of treating, preventing and/or managing cancer as well as and diseases and disorders associated with, or characterized by, undesired angiogenesis are disclosed. Specific methods encompass the administration of an immunomodulatory compound alone or in combination with a second active ingredient. The invention further relates to methods of reducing or avoiding adverse side effects associated with chemotherapy, radiation therapy, hormonal therapy, biological therapy or immunotherapy which comprise the administration of an immunomodulatory compound. Pharmaceutical compositions, single unit dosage forms, and kits suitable for use in methods of the invention are also disclosed.

**13 Claims, 1 Drawing Sheet**

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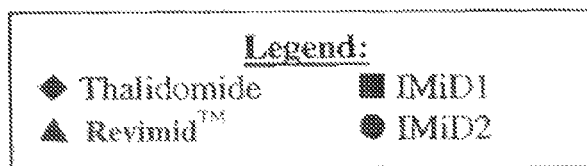
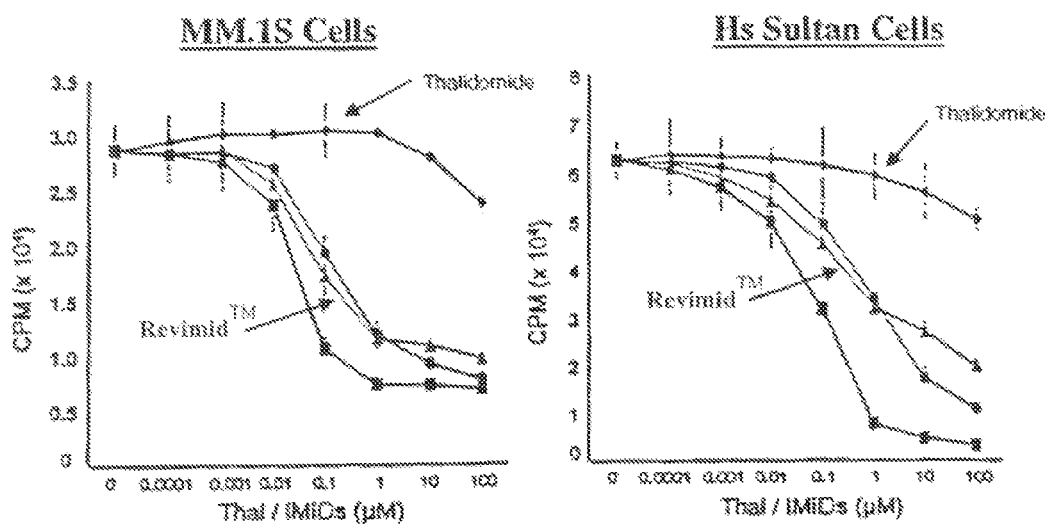
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U.S. Patent

Sep. 10, 2013

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Effects of Revimid<sup>TM</sup> and Thalidomide on MM Cell Proliferation

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**METHODS FOR TREATING MULTIPLE  
MYELOMA WITH  
3-(4-AMINO-1-OXO-1,3-DIHYDROISOINDOL-  
2-YL)PIPERIDINE-2,6-DIONE**

This application is a continuation of U.S. patent application Ser. No. 13/488,888, filed Jun. 5, 2012, which is a continuation of U.S. patent application Ser. No. 12/640,702, filed Dec. 17, 2009, now U.S. Pat. No. 8,198,306, which is a continuation application of U.S. patent application Ser. No. 10/438,213, filed May 15, 2003, now U.S. Pat. No. 7,968,569, which claims the benefit of U.S. provisional application Nos. 60/380,842, filed May 17, 2002, and 60/424,600, filed Nov. 6, 2002, the entireties of which are incorporated herein by reference.

## 1. FIELD OF THE INVENTION

This invention relates to methods of treating, preventing and/or managing specific cancers, and other diseases including, but not limited to, those associated with, or characterized by, undesired angiogenesis, by the administration of one or more immunomodulatory compounds alone or in combination with other therapeutics. In particular, the invention encompasses the use of specific combinations, or “cocktails,” of drugs and other therapy, e.g., radiation to treat these specific cancers, including those refractory to conventional therapy. The invention also relates to pharmaceutical compositions and dosing regimens.

## 2. BACKGROUND OF THE INVENTION

### 2.1 Pathobiology of Cancer and Other Diseases

Cancer is characterized primarily by an increase in the number of abnormal cells derived from a given normal tissue, invasion of adjacent tissues by these abnormal cells, or lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites (metastasis). Clinical data and molecular biologic studies indicate that cancer is a multistep process that begins with minor preneoplastic changes, which may under certain conditions progress to neoplasia. The neoplastic lesion may evolve clonally and develop an increasing capacity for invasion, growth, metastasis, and heterogeneity, especially under conditions in which the neoplastic cells escape the host's immune surveillance. Roitt, I., Brostoff, J and Kale, D., *Immunology*, 17.1-17.12 (3rd ed., Mosby, St. Louis, Mo., 1993).

There is an enormous variety of cancers which are described in detail in the medical literature. Examples includes cancer of the lung, colon, rectum, prostate, breast, brain, and intestine. The incidence of cancer continues to climb as the general population ages, as new cancers develop, and as susceptible populations (e.g., people infected with AIDS or excessively exposed to sunlight) grow. A tremendous demand therefore exists for new methods and compositions that can be used to treat patients with cancer.

Many types of cancers are associated with new blood vessel formation, a process known as angiogenesis. Several of the mechanisms involved in tumor-induced angiogenesis have been elucidated. The most direct of these mechanisms is the secretion by the tumor cells of cytokines with angiogenic properties. Examples of these cytokines include acidic and basic fibroblastic growth factor (a,b-FGF), angiogenin, vascular endothelial growth factor (VEGF), and TNF- $\alpha$ . Alternatively, tumor cells can release angiogenic peptides through the production of proteases and the subsequent breakdown of the extracellular matrix where some cytokines are stored

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(e.g., b-FGF). Angiogenesis can also be induced indirectly through the recruitment of inflammatory cells (particularly macrophages) and their subsequent release of angiogenic cytokines (e.g., TNF- $\alpha$ , bFGF).

A variety of other diseases and disorders are also associated with, or characterized by, undesired angiogenesis. For example, enhanced or unregulated angiogenesis has been implicated in a number of diseases and medical conditions including, but not limited to, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, rubeosis (neovascularization of the angle), viral diseases, genetic diseases, inflammatory diseases, allergic diseases, and autoimmune diseases. Examples of such diseases and conditions include, but are not limited to: diabetic retinopathy; retinopathy of prematurity; corneal graft rejection; neovascular glaucoma; retrolental fibroplasia; and proliferative vitreoretinopathy.

Accordingly, compounds that can control angiogenesis or inhibit the production of certain cytokines, including TNF- $\alpha$ , may be useful in the treatment and prevention of various diseases and conditions.

### 2.2 Methods of Treating Cancer

Current cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient (see, for example, Stockdale, 1998, *Medicine*, vol. 3, Rubenstein and Federman, eds., Chapter 12, Section IV). Recently, cancer therapy could also involve biological therapy or immunotherapy. All of these approaches pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of a patient or may be unacceptable to the patient. Additionally, surgery may not completely remove neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue. Radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent. Although hormonal therapy can be effective, it is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of cancer cells. Biological therapies and immunotherapies are limited in number and may produce side effects such as rashes or swellings, flu-like symptoms, including fever, chills and fatigue, digestive tract problems or allergic reactions.

With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A majority of cancer chemotherapeutics act by inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division. Gilman et al., *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, Tenth Ed. (McGraw Hill, New York).

Despite availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks. Stockdale, *Medicine*, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. 10, 1998. Almost all chemotherapeutic agents are toxic, and chemotherapy causes significant, and often dangerous side effects including severe nausea, bone marrow depression, and immunosuppression. Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even if those agents act by different mechanism from those of the drugs used in the specific treatment. This phenomenon is referred to as pleio-



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tropic drug or multidrug resistance. Because of the drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

Other diseases or conditions associated with, or characterized by, undesired angiogenesis are also difficult to treat. However, some compounds such as protamine, heparin and steroids have been proposed to be useful in the treatment of certain specific diseases. Taylor et al., *Nature* 297:307 (1982); Folkman et al., *Science* 221:719 (1983); and U.S. Pat. Nos. 5,001,116 and 4,994,443. Thalidomide and certain derivatives of it have also been proposed for the treatment of such diseases and conditions. U.S. Pat. Nos. 5,593,990, 5,629,327, 5,712,291, 6,071,948 and 6,114,355 to D'Amato.

Still, there is a significant need for safe and effective methods of treating, preventing and managing cancer and other diseases and conditions, particularly for diseases that are refractory to standard treatments, such as surgery, radiation therapy, chemotherapy and hormonal therapy, while reducing or avoiding the toxicities and/or side effects associated with the conventional therapies.

### 2.3 IMiDS™

A number of studies have been conducted with the aim of providing compounds that can safely and effectively be used to treat diseases associated with abnormal production of TNF- $\alpha$ . See, e.g., Marriott, J. B., et al., *Expert Opin. Biol. Ther.* 1(4):1-8 (2001); G. W. Muller, et al., *Journal of Medicinal Chemistry* 39(17): 3238-3240 (1996); and G. W. Muller, et al., *Bioorganic & Medicinal Chemistry Letters* 8: 2669-2674 (1998). Some studies have focused on a group of compounds selected for their capacity to potentially inhibit TNF- $\alpha$  production by LPS stimulated PBMC. L. G. Corral, et al., *Ann. Rheum. Dis.* 58: (Suppl 1) 1107-1113 (1999). These compounds, which are referred to as IMiDS™ (Celgene Corporation) or Immunomodulatory Drugs, show not only potent inhibition of TNF- $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  and IL12 production. LPS induced IL6 is also inhibited by immunomodulatory compounds, albeit partially. These compounds are potent stimulators of LPS induced IL10. Id. Particular examples of IMiDS™s include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles described in U.S. Pat. Nos. 6,281,230 and 6,316,471, both to G. W. Muller, et al.

### 3. SUMMARY OF THE INVENTION

This invention encompasses methods of treating and preventing certain types of cancer, including primary and metastatic cancer, as well as cancers that are refractory or resistant to conventional chemotherapy. The methods comprise administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. The invention also encompasses methods of managing certain cancers (e.g., preventing or prolonging their recurrence, or lengthening the time of remission) which comprise administering to a patient in need of such management a prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage cancer. Examples of such conventional therapies include, but are

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not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention also encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are associated with, or characterized by, undesired angiogenesis, which comprise administering to a patient in need of such treatment, management or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In other methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage diseases or disorders associated with, or characterized by, undesired angiogenesis. Examples of such conventional therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention encompasses pharmaceutical compositions, single unit dosage forms, dosing regimens and kits which comprise an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second, or additional, active agent. Second active agents include specific combinations, or "cocktails," of drugs.

### 4. BRIEF DESCRIPTION OF FIGURE

FIG. 1 shows a comparison of the effects of 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide in inhibiting the proliferation of multiple myeloma (MM) cell lines in an in vitro study. The uptake of [<sup>3</sup>H]-thymidine by different MM cell lines (MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of the cell proliferation.

### 5. DETAILED DESCRIPTION OF THE INVENTION

A first embodiment of the invention encompasses methods of treating, managing, or preventing cancer which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with another drug ("second active agent") or method of treating, managing, or preventing cancer. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage cancer.

Another embodiment of the invention encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are characterized by undesired angiogenesis. These methods comprise the administration of a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

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Examples of diseases and disorders associated with, or characterized by, undesired angiogenesis include, but are not limited to, inflammatory diseases, autoimmune diseases, viral diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, and rubeosis (neovascularization of the angle).

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with a second active agent or method of treating, managing, or preventing the disease or condition. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage disease and conditions associated with, or characterized by, undesired angiogenesis.

The invention also encompasses pharmaceutical compositions (e.g., single unit dosage forms) that can be used in methods disclosed herein. Particular pharmaceutical compositions comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second active agent.

#### 5.1 Immunomodulatory Compounds

Compounds used in the invention include immunomodulatory compounds that are racemic, stereomerically enriched or stereomerically pure, and pharmaceutically acceptable salts, solvates, hydrates, stereoisomers, clathrates, and prodrugs thereof. Preferred compounds used in the invention are small organic molecules having a molecular weight less than about 1,000 g/mol, and are not proteins, peptides, oligonucleotides, oligosaccharides or other macromolecules.

As used herein and unless otherwise indicated, the terms "immunomodulatory compounds" and "IMiDs™" (Celgene Corporation) encompasses small organic molecules that markedly inhibit TNF- $\alpha$ , LPS induced monocyte IL1 $\beta$  and IL12, and partially inhibit IL6 production. Specific immunomodulatory compounds are discussed below.

TNF- $\alpha$  is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation. TNF- $\alpha$  is responsible for a diverse range of signaling events within cells. TNF- $\alpha$  may play a pathological role in cancer. Without being limited by theory, one of the biological effects exerted by the immunomodulatory compounds of the invention is the reduction of synthesis of TNF- $\alpha$ . Immunomodulatory compounds of the invention enhance the degradation of TNF- $\alpha$  mRNA.

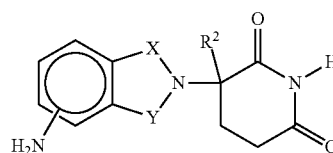
Further, without being limited by theory, immunomodulatory compounds used in the invention may also be potent co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. Immunomodulatory compounds of the invention may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset. In addition, the compounds preferably have anti-inflammatory properties, and efficiently co-stimulate T cells.

Specific examples of immunomodulatory compounds of the invention, include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl)isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl)isoindolines such as those

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described in U.S. Pat. No. 5,874,448; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolines described in U.S. Pat. No. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines (e.g., 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517; and a class of non-polypeptide cyclic amides disclosed in U.S. Pat. Nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S. Pat. Nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles such as those described in U.S. Pat. Nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those described in U.S. patent application Ser. No. 09/972,487 filed on Oct. 5, 2001, U.S. patent application Ser. No. 10/032,286 filed on Dec. 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents and patent applications identified herein are incorporated herein by reference. Immunomodulatory compounds of the invention do not include thalidomide.

Other specific immunomodulatory compounds of the invention include, but are not limited to, 1-oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein by reference. These compounds have the structure I:



in which one of X and Y is C=O, the other of X and Y is C=O or CH<sub>2</sub>, and R<sup>2</sup> is hydrogen or lower alkyl, in particular methyl. Specific immunomodulatory compounds include, but are not limited to:

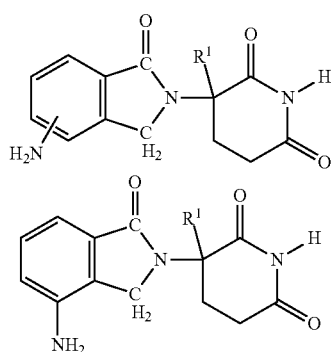
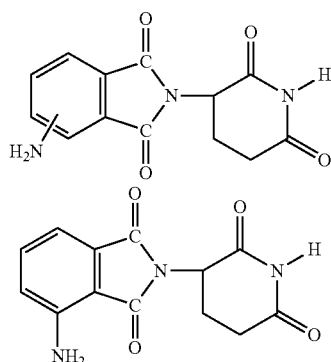
- 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;
- 1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;
- 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;
- 1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;
- 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;
- and
- 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

Other specific immunomodulatory compounds of the invention belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles, such as those described in U.S. Pat. Nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (International Publication No. WO 98/03502), each of which is incorporated herein by reference. Compounds representative of this class are of the formulas:



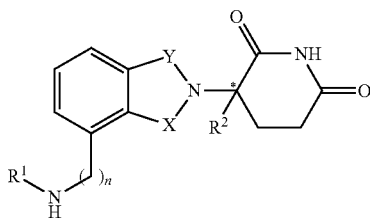
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wherein  $R^1$  is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (e.g. optically pure (R) or (S) enantiomers) of these compounds.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. Nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are incorporated herein by reference. Representative compounds are of formula II:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is  $C=O$  and the other is  $CH_2$  or  $C=O$ ;

$R^1$  is H,  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $C(O)R^3$ ,  $C(S)R^3$ ,  $C(O)OR^4$ ,  $(C_1-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $C(O)NHR^3$ ,  $C(S)NHR^3$ ,  $C(O)NR^3R^{3'}$ ,  $C(S)NR^3R^{3'}$  or  $(C_1-C_8)$ alkyl- $O(CO)R^5$ ;

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$R^2$  is H, F, benzyl,  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl, or  $(C_2-C_8)$ alkynyl;

$R^3$  and  $R^{3'}$  are independently  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_0-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $(C_1-C_8)$ alkyl- $O(CO)R^5$ , or  $C(O)OR^5$ ;

$R^4$  is  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl,  $(C_1-C_4)$ alkyl- $OR^5$ , benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl, or  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl;

$R^5$  is  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl, or  $(C_2-C_5)$ heteroaryl;

each occurrence of  $R^6$  is independently H,  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_2-C_5)$ heteroaryl, or  $(C_0-C_8)$ alkyl- $C(O)OR^5$  or the  $R^6$  groups can join to form a heterocycloalkyl group;

n is 0 or 1; and

\* represents a chiral-carbon center.

In specific compounds of formula II, when n is 0 then  $R^1$  is  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $C(O)R^3$ ,  $C(O)OR^4$ ,  $(C_1-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $C(S)NHR^3$ , or  $(C_1-C_8)$ alkyl- $O(CO)R^5$ ;

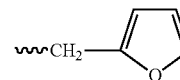
$R^2$  is H or  $(C_1-C_8)$ alkyl; and

$R^3$  is  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_5-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_0-C_8)$ alkyl- $NH-C(O)OR^5$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $(C_1-C_8)$ alkyl- $O(CO)R^5$ , or  $C(O)OR^5$ ; and the other variables have the same definitions.

In other specific compounds of formula II,  $R^2$  is H or  $(C_1-C_4)$ alkyl.

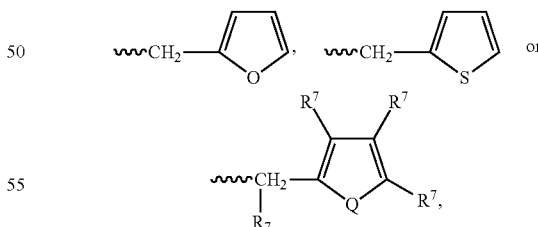
In other specific compounds of formula II,  $R^1$  is  $(C_1-C_8)$ alkyl or benzyl.

In other specific compounds of formula II,  $R^1$  is H,  $(C_1-C_8)$ alkyl, benzyl,  $CH_2OCH_3$ ,  $CH_2CH_2OCH_3$ , or



In another embodiment of the compounds of formula II,  $R^1$  is

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wherein Q is O or S, and each occurrence of  $R^7$  is independently H,  $(C_1-C_8)$ alkyl, benzyl,  $CH_2OCH_3$ , or  $CH_2CH_2OCH_3$ .

In other specific compounds of formula II,  $R^1$  is  $C(O)R^3$ .

In other specific compounds of formula II,  $R^3$  is  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_1-C_8)$ alkyl, aryl, or  $(C_0-C_4)$ alkyl- $OR^5$ .

In other specific compounds of formula II, heteroaryl is pyridyl, furyl, or thienyl.

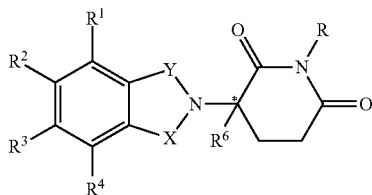
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In other specific compounds of formula II,  $R^1$  is  $C(O)OR^4$ .

In other specific compounds of formula II, the H of  $C(O)NHC(O)$  can be replaced with  $(C_1-C_4)$ alkyl, aryl, or benzyl.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. No. 09/781,179, International Publication No. WO 98/54170, and U.S. Pat. No. 6,395,754, each of which are incorporated herein by reference. Representative compounds are of formula III:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is  $C=O$  and the other is  $CH_2$  or  $C=O$ ;

R is H or  $CH_2OCOR^5$ ;

(i) each of  $R^1$ ,  $R^2$ ,  $R^3$ , or  $R^4$ , independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of  $R^1$ ,  $R^2$ ,  $R^3$ , or  $R^4$  is nitro or  $-NHR^5$  and the remaining of  $R^1$ ,  $R^2$ ,  $R^3$ , or  $R^4$  are hydrogen;

$R^5$  is hydrogen or alkyl of 1 to 8 carbons

$R^6$  hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro;

$R^7$  is  $R^7-CHR^{10}-N(R^8R^9)$ ;

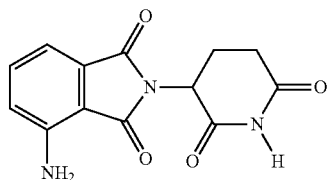
$R^7$  is m-phenylene or p-phenylene or  $-(C_nH_{2n})-$  in which n has a value of 0 to 4;

each of  $R^8$  and  $R^9$  taken independently of the other is hydrogen or alkyl of 1 to 8 carbon atoms, or  $R^8$  and  $R^9$  taken together are tetramethylene, pentamethylene, hexamethylene, or  $-CH_2CH_2[X]X_1CH_2CH_2-$  in which  $[X]X_1$  is  $-O-$ ,  $-S-$ , or  $-NH-$ ;

$R^{10}$  is hydrogen, alkyl of 1 to 8 carbon atoms, or phenyl; and

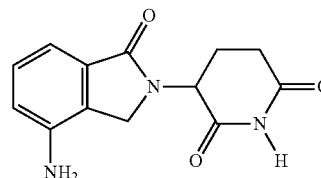
\* represents a chiral-carbon center.

The most preferred immunomodulatory compounds of the invention are 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. The compounds can be obtained via standard, synthetic methods (see e.g., U.S. Pat. No. 5,635, 517, incorporated herein by reference). The compounds are available from Celgene Corporation, Warren, N.J. 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (ACTIMID<sup>TM</sup>) has the following chemical structure:



The compound 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (REVIMID<sup>TM</sup>) has the following chemical structure:

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Compounds of the invention can either be commercially purchased or prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry techniques.

As used herein and unless otherwise indicated, the term "pharmaceutically acceptable salt" encompasses non-toxic acid and base addition salts of the compound to which the term refers. Acceptable non-toxic acid addition salts include those derived from organic and inorganic acids or bases known in the art, which include, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methane-sulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embolic acid, enanthic acid, and the like.

Compounds that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds are those that form non-toxic base addition salts, i.e., salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzyl-ethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), lysine, and procaine.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of immunomodulatory compounds of the invention that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of immunomodulatory compounds of the invention that comprise  $-NO$ ,  $-NO_2$ ,  $-ONO$ , or  $-ONO_2$  moieties. Prodrugs can typically be prepared using well-known methods, such as those described in 1 *Burger's Medicinal Chemistry and Drug Discovery*, 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995), and *Design of Prodrugs* (H. Bundgaard ed., Elsevier, New York 1985).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologi-

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cally inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxyethyl, acetoxyethyl, aminocarbonyloxyethyl, pivaloyloxyethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyl-oxyethyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides,  $\alpha$ -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, amino acids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

Various immunomodulatory compounds of the invention contain one or more chiral centers, and can exist as racemic mixtures of enantiomers or mixtures of diastereomers. This invention encompasses the use of stereomerically pure forms of such compounds, as well as the use of mixtures of those forms. For example, mixtures comprising equal or unequal amounts of the enantiomers of a particular immunomodulatory compounds of the invention may be used in methods and compositions of the invention. These isomers may be asymmetrically synthesized or resolved using standard techniques such as chiral columns or chiral resolving agents. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. As used herein and unless otherwise indicated, the term "stereomerically enriched" means a composition that comprises greater than about 60% by weight of one stereoisomer of a compound, preferably greater than about 70% by weight, more preferably greater than about 80% by weight of one stereoisomer of a compound. As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center. Similarly, the term "stereomerically enriched" means a stereomerically enriched composition of a compound having one chiral center.

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It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

## 5.2 Second Active Agents

Immunomodulatory compounds can be combined with other pharmacologically active compounds ("second active agents") in methods and compositions of the invention. It is believed that certain combinations work synergistically in the treatment of particular types of cancer and certain diseases and conditions associated with, or characterized by, undesired angiogenesis. Immunomodulatory compounds can also work to alleviate adverse effects associated with certain second active agents, and some second active agents can be used to alleviate adverse effects associated with immunomodulatory compounds.

One or more second active ingredients or agents can be used in the methods and compositions of the invention together with an immunomodulatory compound. Second active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules).

Examples of large molecule active agents include, but are not limited to, hematopoietic growth factors, cytokines, and monoclonal and polyclonal antibodies. Typical large molecule active agents are biological molecules, such as naturally occurring or artificially made proteins. Proteins that are particularly useful in this invention include proteins that stimulate the survival and/or proliferation of hematopoietic precursor cells and immunologically active poietic cells in vitro or in vivo. Others stimulate the division and differentiation of committed erythroid progenitors in cells in vitro or in vivo. Particular proteins include, but are not limited to: interleukins, such as IL-2 (including recombinant IL-II ("rIL2") and canarypox TL-2), IL-10, IL-12, and IL-18; interferons, such as interferon alfa-2a, interferon alfa-2b, interferon alfa-n1, interferon alfa-n3, interferon beta-1 a, and interferon gamma-I b; GM-CSF and GM-CSF; and EPO.

Particular proteins that can be used in the methods and compositions of the invention include, but are not limited to: filgrastim, which is sold in the United States under the trade name Neupogen® (Amgen, Thousand Oaks, Calif.); sargramostim, which is sold in the United States under the trade name Leukine® (Immunex, Seattle, Wash.); and recombinant EPO, which is sold in the United States under the trade name Epogen® (Amgen, Thousand Oaks, Calif.).

Recombinant and mutated forms of GM-CSF can be prepared as described in U.S. Pat. Nos. 5,391,485; 5,393,870; and 5,229,496; all of which are incorporated herein by reference. Recombinant and mutated forms of G-CSF can be prepared as described in U.S. Pat. Nos. 4,810,643; 4,999,291; 5,528,823; and 5,580,755; all of which are incorporated herein by reference.

This invention encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term "mutants" are proteins that lack carbohydrate moieties normally present in their naturally occurring forms

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(e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. J. Immunol. Methods* 248:91-101 (2001).

Antibodies that can be used in combination with compounds of the invention include monoclonal and polyclonal antibodies. Examples of antibodies include, but are not limited to, trastuzumab (Herceptin®), rituximab (Rituxan®), bevacizumab (Avastin™), pertuzumab (Omnitarg™), tositumomab (Bexxar®), edrecolomab (Panorex®), and G250. Compounds of the invention can also be combined with, or used in combination with, anti-TNF- $\alpha$  antibodies.

Large molecule active agents may be administered in the form of anti-cancer vaccines. For example, vaccines that secrete, or cause the secretion of, cytokines such as IL-2, G-CSF, and GM-CSF can be used in the methods, pharmaceutical compositions, and kits of the invention. See, e.g., Emens, L. A., et al., *Curr. Opinion Mol. Ther.* 3(1):77-84 (2001).

In one embodiment of the invention, the large molecule active agent reduces, eliminates, or prevents an adverse effect associated with the administration of an immunomodulatory compound. Depending on the particular immunomodulatory compound and the disease or disorder being treated, adverse effects can include, but are not limited to, drowsiness and somnolence, dizziness and orthostatic hypotension, neutropenia, infections that result from neutropenia, increased HIV-viral load, bradycardia, Stevens-Johnson Syndrome and toxic epidermal necrolysis, and seizures (e.g., grand mal convulsions). A specific adverse effect is neutropenia.

Second active agents that are small molecules can also be used to alleviate adverse effects associated with the administration of an immunomodulatory compound. However, like some large molecules, many are believed to be capable of providing a synergistic effect when administered with (e.g., before, after or simultaneously) an immunomodulatory compound. Examples of small molecule second active agents include, but are not limited to, anti-cancer agents, antibiotics, immunosuppressive agents, and steroids.

Examples of anti-cancer agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; celecoxib (COX-2 inhibitor); chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; iproplatin; irinotecan; irinotecan

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hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprostol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; pipsulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procabazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiro-mustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; taxotere; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene acetate; tretolone acetate; tricitriline phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; and zorubicin hydrochloride.

Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamcstane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin ITT derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betacalamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; capecitabine; carboxamide-amino-triazole; carboxamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroquinoline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conage-nin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatin; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrididemnin B; deslorelin; dexamethasone; dexifosfamide; dextrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; doxorubicin; droloxifene; dronabinol; duocarmycin SA; ebsclen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride;



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estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilomofosine; ilomastat; imatinib (e.g., Gleevec®), imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostin; mitoguanzone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; Erbitux, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullin; oblimersen (Genasense®); O<sup>6</sup>-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiriomycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin;

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spongistatin 1; squalamine; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; tallimustine; tamoxifen methiodide; taumustine; tazartene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; velaresol; veramine; verdins; verteporfin; vinorelbine; vinoxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Specific second active agents include, but are not limited to, oblimersen (Genasense®), remicade, docetaxel, celecoxib, melphalan, dexamethasone (Decadron®), steroids, gemcitabine, cisplatin, temozolomide, etoposide, cyclophosphamide, temodar, carboplatin, procarbazine, gliadel, tamoxifen, topotecan, methotrexate, Arisa®, taxol, taxotere, fluorouracil, leucovorin, irinotecan, xeloda, CPT-11, interferon alpha, pegylated interferon alpha (e.g., PEG INTRON-A), capecitabine, cisplatin, thiotepa, fludarabine, carboplatin, liposomal daunorubicin, cytarabine, doxorubicin, paclitaxel, vinblastine, IL-2, GM-CSF, dacarbazine, vinorelbine, zoledronic acid, palmitronate, biaxin, busulphan, prednisone, bisphosphonate, arsenic trioxide, vincristine, doxorubicin (Doxil®), paclitaxel, ganciclovir, adriamycin, estramustine sodium phosphate (Emcyt®), sulindac, and etoposide.

### 5.3 Methods of Treatments and Prevention

Methods of this invention encompass methods of treating, preventing and/or managing various types of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis. As used herein, unless otherwise specified, the term “treating” refers to the administration of a compound of the invention or other additional active agent after the onset of symptoms of the particular disease or disorder. As used herein, unless otherwise specified, the term “preventing” refers to the administration prior to the onset of symptoms, particularly to patients at risk of cancer, and other diseases and disorders associated with, or characterized by, undesired angiogenesis. The term “prevention” includes the inhibition of a symptom of the particular disease or disorder. Patients with familial history of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis are preferred candidates for preventive regimens. As used herein and unless otherwise indicated, the term “managing” encompasses preventing the recurrence of the particular disease or disorder in a patient who had suffered from it, and/or lengthening the time a patient who had suffered from the disease or disorder remains in remission.

As used herein, the term “cancer” includes, but is not limited to, solid tumors and blood born tumors. The term “cancer” refers to disease of skin tissues, organs, blood, and vessels, including, but not limited to, cancers of the bladder, bone or blood, brain, breast, cervix, chest, colon, endometrium, esophagus, eye, head, kidney, liver, lymph nodes, lung, mouth, neck, ovaries, pancreas, prostate, rectum, stomach, testis, throat, and uterus. Specific cancers include, but are not limited to, advanced malignancy, amyloidosis, neuroblastoma, meningioma, hemangiopericytoma, multiple brain metastase, glioblastoma multiforms, glioblastoma, brain stem glioma, poor prognosis malignant brain tumor,

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malignant glioma, recurrent malignant glioma, anaplastic astrocytoma, anaplastic oligodendroglioma, neuroendocrine tumor, rectal adenocarcinoma, Dukes C & D colorectal cancer, unresectable colorectal carcinoma, metastatic hepatocellular carcinoma, Kaposi's sarcoma, karotype acute myeloblastic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma, low grade follicular lymphoma, malignant melanoma, malignant mesothelioma, malignant pleural effusion mesothelioma syndrome, peritoneal carcinoma, papillary serous carcinoma, gynecologic sarcoma, soft tissue sarcoma, scleroderma, cutaneous vasculitis, Langerhans cell histiocytosis, leiomyosarcoma, fibrodysplasia ossificans progressive, hormone refractory prostate cancer, resected high-risk soft tissue sarcoma, unresectable hepatocellular carcinoma, Waldenstrom's macroglobulinemia, smoldering myeloma, indolent myeloma, fallopian tube cancer, androgen independent prostate cancer, androgen dependent stage IV non-metastatic prostate cancer, hormone-insensitive prostate cancer, chemotherapy-insensitive prostate cancer, papillary thyroid carcinoma, follicular thyroid carcinoma, medullary thyroid carcinoma, and leiomyoma. In a specific embodiment, the cancer is metastatic. In another embodiment, the cancer is refractory or resistance to chemotherapy or radiation; in particular, refractory to thalidomide.

As used herein to refer to diseases and conditions other than cancer, the terms "diseases or disorders associated with, or characterized by, undesired angiogenesis," "diseases or disorders associated with undesired angiogenesis," and "diseases or disorders characterized by undesired angiogenesis" refer to diseases, disorders and conditions that are caused, mediated or attended by undesired, unwanted or uncontrolled angiogenesis, including, but not limited to, inflammatory diseases, autoimmune diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroidal neovascular diseases, and retina neovascular diseases.

Examples of such diseases or disorders associated with undesired angiogenesis include, but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, proliferative vitreoretinopathy, trachoma, myopia, optic pits, epidemic keratoconjunctivitis, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, syphilis, lipid degeneration, bacterial ulcer, fungal ulcer, Herpes simplex infection, Herpes zoster infection, protozoan infection, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, peripheral radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Behcet's disease, retinitis, choroiditis, presumed ocular histoplasmosis, Bests disease, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, rubeosis, sarcoidosis, sclerosis, soriatis, psoriasis, primary sclerosing cholangitis, proctitis, primary biliary srosis, idiopathic pulmonary fibrosis, and alcoholic hepatitis.

In specific embodiments of the invention, diseases or disorders associated with undesired angiogenesis do not include congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, myocardial infarction, HIV, hepatitis, adult respiratory distress syndrome, bone-resorption disease, chronic obstructive pulmonary diseases, chronic pul-

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monary inflammatory disease, dermatitis, cystic fibrosis, septic shock, sepsis, endotoxic shock, hemodynamic shock, sepsis syndrome, post ischemic reperfusion injury, meningitis, psoriasis, fibrotic disease, cachexia, graft rejection, rheumatoid spondylitis, osteoporosis, Crohn's disease, ulcerative colitis, inflammatory-bowel disease, multiple sclerosis, systemic lupus erythematosus, erythema nodosum leprosum in leprosy, radiation damage, asthma, hyperoxic alveolar injury, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

This invention encompasses methods of treating patients who have been previously treated for cancer or diseases or disorders associated with, or characterized by, undesired angiogenesis, but are non-responsive to standard therapies, as well as those who have not previously been treated. The invention also encompasses methods of treating patients regardless of patient's age, although some diseases or disorders are more common in certain age groups. The invention further encompasses methods of treating patients who have undergone surgery in an attempt to treat the disease or condition at issue, as well as those who have not. Because patients with cancer and diseases and disorders characterized by undesired angiogenesis have heterogenous clinical manifestations and varying clinical outcomes, the treatment given to a patient may vary, depending on his/her prognosis. The skilled clinician will be able to readily determine without undue experimentation specific secondary agents, types of surgery, and types of non-drug based standard therapy that can be effectively used to treat an individual patient with cancer and other diseases or disorders.

Methods encompassed by this invention comprise administering one or more immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, to a patient (e.g., a human) suffering, or likely to suffer, from cancer or a disease or disorder mediated by undesired angiogenesis.

In one embodiment of the invention, an immunomodulatory compound of the invention can be administered orally in single or divided daily doses in an amount of from about 0.10 to about 150 mg/day. In a particular embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of from about 0.1 to about 1 mg per day, or alternatively from about 0.1 to about 5 mg every other day. In a preferred embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered in an amount of from about 5 to 25 mg per day, or alternatively from about 10 to about 50 mg every other day.

In a specific embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of about 1, 2, or 5 mg per day to patients with relapsed multiple myeloma. In a particular embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered initially in an amount of 5 mg/day and the dose can be escalated every week to 10, 20, 25, 30 and 50 mg/day. In a specific embodiment, Revimid™ can be administered in an amount of up to about 30 mg/day to patients with solid tumor. In a particular embodiment, Revimid™ can be administered in an amount of up to about 40 mg/day to patients with glioma.

#### 5.3.1 Combination Therapy with a Second Active Agent

Specific methods of the invention comprise administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with one or more second active agents, and/or in combination with radia-

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tion therapy, blood transfusions, or surgery. Examples of immunomodulatory compounds of the invention are disclosed herein (see, e.g., section 5.1). Examples of second active agents are also disclosed herein (see, e.g., section 5.2).

Administration of the immunomodulatory compounds and the second active agents to a patient can occur simultaneously or sequentially by the same or different routes of administration. The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. A preferred route of administration for an immunomodulatory compound of the invention is orally. Preferred routes of administration for the second active agents or ingredients of the invention are known to those of ordinary skill in the art. See, e.g., *Physicians' Desk Reference*, 1755-1760 (56<sup>th</sup> ed., 2002).

In one embodiment of the invention, the second active agent is administered intravenously or subcutaneously and once or twice daily in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. The specific amount of the second active agent will depend on the specific agent used, the type of disease being treated or managed, the severity and stage of disease, and the amount(s) of immunomodulatory compounds of the invention and any optional additional active agents concurrently administered to the patient. In a particular embodiment, the second active agent is oblimersen (Genasense®), GM-CSF, G-CSF, EPO, taxotere, irinotecan, dacarbazine, transretinoic acid, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, vincristine, doxorubicin, COX-2 inhibitor, IL2, IL8, IL18, IFN, Ara-C, vinorelbine, or a combination thereof.

In a particular embodiment, GM-CSF, G-CSF or EPO is administered subcutaneously during about five days in a four or six week cycle in an amount of from about 1 to about 750 mg/m<sup>2</sup>/day, preferably in an amount of from about 25 to about 500 mg/m<sup>2</sup>/day, more preferably in an amount of from about 50 to about 250 mg/m<sup>2</sup>/day, and most preferably in an amount of from about 50 to about 200 mg/m<sup>2</sup>/day. In a certain embodiment, GM-CSF may be administered in an amount of from about 60 to about 500 mcg/m<sup>2</sup> intravenously over 2 hours, or from about 5 to about 12 mcg/m<sup>2</sup>/day subcutaneously. In a specific embodiment, G-CSF may be administered subcutaneously in an amount of about 1 mcg/kg/day initially and can be adjusted depending on rise of total granulocyte counts. The maintenance dose of G-CSF may be administered in an amount of about 300 (in smaller patients) or 480 mcg subcutaneously. In a certain embodiment, EPO may be administered subcutaneously in an amount of 10,000 Unit 3 times per week.

In another embodiment, Revimid™ in an amount of about 25 mg/d and dacarbazine in an amount of about from 200 to 1,000 mg/m<sup>2</sup>/d are administered to patients with metastatic malignant melanoma. In a specific embodiment, Revimid™ is administered in an amount of from about 5 to about 25 mg/d to patients with metastatic malignant melanoma whose disease has progressed on treatment with dacarbazine, IL-2 or IFN. In a specific embodiment, Revimid™ is administered to patients with relapsed or refractory multiple myeloma in an amount of about 15 mg/d twice a day or about 30 mg/d four times a day in a combination with dexamethasone.

In another embodiment, an immunomodulatory compound is administered with melphalan and dexamethasone to patients with amyloidosis. In a specific embodiment, an immunomodulatory compound of the invention and steroids can be administered to patients with amyloidosis.

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In another embodiment, an immunomodulatory compound is administered with gemcitabine and cisplatin to patients with locally advanced or metastatic transitional cell bladder cancer.

In another embodiment, an immunomodulatory compound is administered in combination with a second active ingredient as follows: temozolomide to pediatric patients with relapsed or progressive brain tumors or recurrent neuroblastoma; celecoxib, etoposide and cyclophosphamide for relapsed or progressive CNS cancer; temodar to patients with recurrent or progressive meningioma, malignant meningioma, hemangiopericytoma, multiple brain metastases, relapsed brain tumors, or newly diagnosed glioblastoma multiformis; irinotecan to patients with recurrent glioblastoma; carboplatin to pediatric patients with brain stem glioma; procarbazine to pediatric patients with progressive malignant gliomas; cyclophosphamide to patients with poor prognosis malignant brain tumors, newly diagnosed or recurrent glioblastoma multiformis; Gliadel® for high grade recurrent malignant gliomas; temozolomide and tamoxifen for anaplastic astrocytoma; or topotecan for gliomas, glioblastoma, anaplastic astrocytoma or anaplastic oligodendroglioma.

In another embodiment, an immunomodulatory compound is administered with methotrexate and cyclophosphamide to patients with metastatic breast cancer.

In another embodiment, an immunomodulatory compound is administered with temozolomide to patients with neuroendocrine tumors.

In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with recurrent or metastatic head or neck cancer. In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with pancreatic cancer.

In another embodiment, an immunomodulatory compound is administered to patients with colon cancer in combination with Arisa®, taxol and/or taxotere.

In another embodiment, an immunomodulatory compound is administered with capecitabine to patients with refractory colorectal cancer or patients who fail first line therapy or have poor performance in colon or rectal adenocarcinoma.

In another embodiment, an immunomodulatory compound is administered in combination with fluorouracil, leucovorin, and irinotecan to patients with Dukes C & D colorectal cancer or to patients who have been previously treated for metastatic colorectal cancer.

In another embodiment, an immunomodulatory compound is administered to patients with refractory colorectal cancer in combination with capecitabine, xeloda, and/or CPT-11.

In another embodiment, an immunomodulatory compound of the invention is administered with capecitabine and irinotecan to patients with refractory colorectal cancer or to patients with unresectable or metastatic colorectal carcinoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with interferon alpha or capecitabine to patients with unresectable or metastatic hepatocellular carcinoma; or with cisplatin and thiopeta to patients with primary or metastatic liver cancer.

In another embodiment, an immunomodulatory compound is administered in combination with pegylated interferon alpha to patients with Kaposi's sarcoma.

In another embodiment, an immunomodulatory compound is administered in combination with fludarabine, carboplatin, and/or topotecan to patients with refractory or relapsed or high-risk acuted myelogenous leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with liposomal daunorubicin,



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topotecan and/or cytarabine to patients with unfavorable karyotype acute myeloblastic leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with gemcitabine and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered in combination with carboplatin and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered with doxetaxol to patients with non-small cell lung cancer who have been previously treated with carbo/VP 16 and radiotherapy.

In another embodiment, an immunomodulatory compound is administered in combination with carboplatin and/or taxotere, or in combination with carboplatin, paclitaxel and/or thoracic radiotherapy to patients with non-small cell lung cancer. In a specific embodiment, an immunomodulatory compound is administered in combination with taxotere to patients with stage IIIB or IV non-small cell lung cancer.

In another embodiment, an immunomodulatory compound of the invention is administered in combination with oblimersen (Genasense®) to patients with small cell lung cancer.

In another embodiment, an immunomodulatory compound is administered alone or in combination with a second active ingredient such as vinblastine or fludarabine to patients with various types of lymphoma, including, but not limited to, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma or relapsed or refractory low grade follicular lymphoma.

In another embodiment, an immunomodulatory compound is administered in combination with taxotere, IL-2, IFN, GM-CSF, and/or dacarbazine to patients with various types or stages of melanoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with vinorelbine to patients with malignant mesothelioma, or stage IIIB non-small cell lung cancer with pleural implants or malignant pleural effusion mesothelioma syndrome.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of multiple myeloma in combination with dexamethasone, zoledronic acid, palmitronate, GM-CSF, biacin, vinblastine, melphalan, busulphan, cyclophosphamide, IFN, palmidronate, prednisone, bisphosphonate, celecoxib, arsenic trioxide, PEG INTRON-A, vincristine, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with relapsed or refractory multiple myeloma in combination with doxorubicin (Doxil®), vincristine and/or dexamethasone (Decadron®).

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of ovarian cancer such as peritoneal carcinoma, papillary serous carcinoma, refractory ovarian cancer or recurrent ovarian cancer, in combination with taxol, carboplatin, doxorubicin, gemcitabine, cisplatin, xeloda, paclitaxel, dexamethasone, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of prostate cancer, in combination with xeloda, 5 FU/LV, gemcitabine, irinotecan plus gemcitabine, cyclophosphamide, vincristine, dexamethasone, GM-CSF, celecoxib, taxotere, ganciclovir, paclitaxel, adriamycin, docetaxel, estramustine, Emcyt, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of

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renal cell cancer, in combination with capecitabine, IFN, tamoxifen, IL-2, GM-CSF, Celebrex®, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of gynecologic, uterus or soft tissue sarcoma cancer in combination with IFN, a COX-2 inhibitor such as Celebrex®, and/or sulindac.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of solid tumors in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apecitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with scleroderma or cutaneous vasculitis in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apecitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

This invention also encompasses a method of increasing the dosage of an anti-cancer drug or agent that can be safely and effectively administered to a patient, which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable derivative, salt, solvate, clathrate, hydrate, or pro-drug thereof. Patients that can benefit by this method are those likely to suffer from an adverse effect associated with anti-cancer drugs for treating a specific cancer of the skin, subcutaneous tissue, lymph nodes, brain, lung, liver, bone, intestine, colon, heart, pancreas, adrenal, kidney, prostate, breast, colorectal, or combinations thereof. The administration of an immunomodulatory compound of the invention alleviates or reduces adverse effects which are of such severity that it would otherwise limit the amount of anti-cancer drug.

In one embodiment, an immunomodulatory compound of the invention can be administered orally and daily in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 50 mg, more preferably from about 2 to about 25 mg prior to, during, or after the occurrence of the adverse effect associated with the administration of an anti-cancer drug to a patient. In a particular embodiment, an immunomodulatory compound of the invention is administered in combination with specific agents such as heparin, aspirin, coumadin, or G-CSF to avoid adverse effects that are associated with anti-cancer drugs such as but not limited to neutropenia or thrombocytopenia.

In one embodiment, an immunomodulatory compound of the invention can be administered to patients with diseases and disorders associated with, or characterized by, undesired angiogenesis in combination with additional active ingredients including but not limited to anti-cancer drugs, anti-inflammatories, antihistamines, antibiotics, and steroids.

In another embodiment, this invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with (e.g. before, during, or after) conventional therapy including, but not limited to, surgery, immunotherapy, biological therapy, radiation therapy, or other non-drug based therapy presently used to treat, prevent or manage cancer. The combined use of the immunomodulatory compounds of the invention and conventional therapy may provide a unique treatment regimen that is unexpectedly effective in certain patients. Without being limited by theory, it is believed that immunomodulatory compounds of the invention may provide additive or synergistic effects when given concurrently with conventional therapy.

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As discussed elsewhere herein, the invention encompasses a method of reducing, treating and/or preventing adverse or undesired effects associated with conventional therapy including, but not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy. One or more immunomodulatory compounds of the invention and other active ingredient can be administered to a patient prior to, during, or after the occurrence of the adverse effect associated with conventional therapy.

In one embodiment, an immunomodulatory compound of the invention can be administered in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 25 mg, more preferably from about 2 to about 10 mg orally and daily alone, or in combination with a second active agent disclosed herein (see, e.g., section 5.2), prior to, during, or after the use of conventional therapy.

In a specific embodiment of this method, an immunomodulatory compound of the invention and doxorubicin are administered to patients with non-small cell lung cancer who were previously treated with carboplatin and radiotherapy.

### 5.3.2 Use with Transplantation Therapy

Compounds of the invention can be used to reduce the risk of Graft Versus Host Disease (GVHD). Therefore, the invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering the immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with transplantation therapy.

As those of ordinary skill in the art are aware, the treatment of cancer is often based on the stages and mechanism of the disease. For example, as inevitable leukemic transformation develops in certain stages of cancer, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. The combined use of the immunomodulatory compound of the invention and transplantation therapy provides a unique and unexpected synergism. In particular, an immunomodulatory compound of the invention exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy in patients with cancer.

An immunomodulatory compound of the invention can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of GVHD. This invention encompasses a method of treating, preventing and/or managing cancer which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after the transplantation of umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow. Examples of stem cells suitable for use in the methods of the invention are disclosed in U.S. provisional patent application No. 60/372,348, filed Apr. 12, 2002 by R. Hariri et al., the entirety of which is incorporated herein by reference.

In one embodiment of this method, an immunomodulatory compound of the invention is administered to patients with multiple myeloma before, during, or after the transplantation of autologous peripheral blood progenitor cell.

In another embodiment, an immunomodulatory compound is administered to patients with relapsing multiple myeloma after the stem cell transplantation.

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In another embodiment, an immunomodulatory compound and prednisone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous stem cell.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as salvage therapy for low risk post transplantation to patients with multiple myeloma.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous bone marrow.

In another embodiment, an immunomodulatory compound is administered following the administration of high dose of melphalan and the transplantation of autologous stem cell to patients with chemotherapy responsive multiple myeloma.

In another embodiment, an immunomodulatory compound and PEG INTRO-A are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous CD34-selected peripheral stem cell.

In another embodiment, an immunomodulatory compound is administered with post transplant consolidation chemotherapy to patients with newly diagnosed multiple myeloma to evaluate anti-angiogenesis.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy after DCEP consolidation, following the treatment with high dose of melphalan and the transplantation of peripheral blood stem cell to 65 years of age or older patients with multiple myeloma.

### 5.3.3 Cycling Therapy

In certain embodiments, the prophylactic or therapeutic agents of the invention are cyclically administered to a patient. Cycling therapy involves the administration of an active agent for a period of time, followed by a rest for a period of time, and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

Consequently, in one specific embodiment of the invention, an immunomodulatory compound of the invention is administered daily in a single or divided doses in a four to six week cycle with a rest period of about a week or two weeks. The invention further allows the frequency, number, and length of dosing cycles to be increased. Thus, another specific embodiment of the invention encompasses the administration of an immunomodulatory compound of the invention for more cycles than are typical when it is administered alone. In yet another specific embodiment of the invention, an immunomodulatory compound of the invention is administered for a greater number of cycles that would typically cause dose-limiting toxicity in a patient to whom a second active ingredient is not also being administered.

In one embodiment, an immunomodulatory compound of the invention is administered daily and continuously for three or four weeks at a dose of from about 0.1 to about 150 mg/d followed by a break of one or two weeks. Actimid™ is preferably administered daily and continuously at an initial dose of 0.1 to 5 mg/d with dose escalation (every week) by 1 to 10 mg/d to a maximum dose of 50 mg/d for as long as therapy is tolerated. In a particular embodiment, Revimid™ is administered in an amount of about 5, 10, or 25 mg/day, preferably in an amount of about 10 mg/day for three to four weeks, followed by one week or two weeks of rest in a four or six week cycle.

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In one embodiment of the invention, an immunomodulatory compound of the invention and a second active ingredient are administered orally, with administration of an immunomodulatory compound of the invention occurring 30 to 60 minutes prior to a second active ingredient, during a cycle of four to six weeks. In another embodiment of the invention, the combination of an immunomodulatory compound of the invention and a second active ingredient is administered by intravenous infusion over about 90 minutes every cycle. In a specific embodiment, one cycle comprises the administration of from about 10 to about 25 mg/day of Revimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient daily for three to four weeks and then one or two weeks of rest. In another specific embodiment, each cycle comprises the administration of from about 5 to about 10 mg/day of Actimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient for 3 to 4 weeks followed by one or two weeks of rest. Typically, the number of cycles during which the combinatorial treatment is administered to a patient will be from about one to about 24 cycles, more typically from about two to about 16 cycles, and even more typically from about four to about three cycles.

#### 5.4 Pharmaceutical Compositions and Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Pharmaceutical compositions and dosage forms of the invention can further comprise one or more excipients.

Pharmaceutical compositions and dosage forms of the invention can also comprise one or more additional active ingredients. Consequently, pharmaceutical compositions and dosage forms of the invention comprise the active ingredients disclosed herein (e.g., an immunomodulatory compound and a second active agent). Examples of optional second, or additional, active ingredients are disclosed herein (see, e.g., section 5.2).

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), topical (e.g., eye drops or other ophthalmic preparations), transdermal or transcutaneous administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; eye drops or other ophthalmic preparations suitable for topical administration; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease. These and other ways in which

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specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients may be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose other mono- or disaccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the *U.S. Pharmacopeia* (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples



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of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as “stabilizers,” include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of from about 0.10 to about 150 mg. Typical dosage forms comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of about 0.1, 1, 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 50, 100, 150 or 200 mg. In a particular embodiment, a preferred dosage form comprises 4-(amino-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) in an amount of about 1, 2, 5, 10, 25 or 50 mg. In a specific embodiment, a preferred dosage form comprises 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) in an amount of about 5, 10, 25 or 50 mg. Typical dosage forms comprise the second active ingredient in an amount of 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. Of course, the specific amount of the anti-cancer drug will depend on the specific agent used, the type of cancer being treated or managed, and the amount(s) of an immunomodulatory compound of the invention and any optional additional active agents concurrently administered to the patient.

#### 5.4.1 Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and inti-

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ately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, PA), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene

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glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

A preferred solid oral dosage form of the invention comprises an immunomodulatory compound of the invention, anhydrous lactose, microcrystalline cellulose, polyvinylpyrrolidone, stearic acid, colloidal anhydrous silica, and gelatin.

#### 5.4.2 Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

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#### 5.4.3 Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention. For example, cyclodextrin and its derivatives can be used to increase the solubility of an immunomodulatory compound of the invention and its derivatives. See, e.g., U.S. Pat. No. 5,134,127, which is incorporated herein by reference.

#### 5.4.4 Topical and Mucosal Dosage Forms

Topical and mucosal dosage forms of the invention include, but are not limited to, sprays, aerosols, solutions, emulsions, suspensions, eye drops or other ophthalmic preparations, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide topical and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form solutions, emulsions or gels, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990).

The pH of a pharmaceutical composition or dosage form may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-

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enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

#### 5.4.5 Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a dosage form of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, pro-drug, or clathrate thereof. Kits encompassed by this invention can further comprise additional active ingredients such as oblimersen (Genasense®), melphalan, G-CSF, GM-CSF, EPO, topotecan, dacarbazine, irinotecan, taxotere, IFN, COX-2 inhibitor, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13 cis-retinoic acid, or a pharmacologically active mutant or derivative thereof, or a combination thereof. Examples of the additional active ingredients include, but are not limited to, those disclosed herein (see, e.g., section 5.2).

Kits of the invention can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

## 6. EXAMPLES

Certain embodiments of the invention are illustrated by the following non-limiting examples.

### 6.1 Modulation of Cytokine Production

A series of non-clinical pharmacology and toxicology studies have been performed to support the clinical evaluation of an immunomodulatory compound of the invention in human subjects. These studies were performed in accordance with internationally recognized guidelines for study design and in compliance with the requirements of Good Laboratory Practice (GLP), unless otherwise noted.

Inhibition of TNF- $\alpha$  production following LPS-stimulation of human PBMC and human whole blood by 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™), 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione and thalidomide (Revimid™) was investigated in vitro (Muller et al., *Bioorg. Med. Chem. Lett.* 9:1625-1630, 1999). The IC<sub>50</sub>'s of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and

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human whole blood were ~24 nM (6.55 ng/mL) and ~25 nM (6.83 ng/mL), respectively. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but at least 200 times more potent than, thalidomide. In vitro studies have also demonstrated that concentrations of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione of 2.73 to 27.3 ng/mL (0.01 to 0.1  $\mu$ M) achieved 50% inhibition of the proliferation of MM.1S and Hs Sultan cells.

The IC<sub>50</sub>'s of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and human whole blood were ~100 nM (25.9 ng/mL) and ~480 nM (103.6 ng/mL), respectively. Thalidomide, in contrast, had an IC<sub>50</sub> of ~194  $\mu$ M (50.2  $\mu$ g/mL) for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but 50 to 2000 times more potent than, thalidomide. It has been shown that the compound is approximately 50-100 times more potent than thalidomide in stimulating the proliferation of T-cells following primary induction by T-cell receptor (TCR) activation. 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is also approximately 50 to 100 times more potent than thalidomide in augmenting the production of IL-2 and IFN- $\gamma$  following TCR activation of PBMC (IL-2) or T-cells (IFN- $\gamma$ ). In addition, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione exhibited dose-dependent inhibition of LPS-stimulated production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 by PBMC while it increased production of the anti-inflammatory cytokine IL-10.

### 6.2 Inhibition of MM Cell Proliferation

The ability of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide for comparison to effect the proliferation of MM cell lines has been investigated in an in vitro study. Uptake [<sup>3</sup>H]-thymidine by different MM cell lines (MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of cell proliferation. Cells were incubated in the presence of compounds for 48 hours; [<sup>3</sup>H]-thymidine was included for the last 8 hours of the incubation period. Addition of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione to MM.1S and Hs Sultan cells resulted in 50% inhibition of cell proliferation at concentrations of 0.4  $\mu$ M and 1  $\mu$ M, respectively. In contrast, addition of thalidomide at concentrations up to 100  $\mu$ M resulted in only 15% and 20% inhibition of cell proliferation in MM.1S and Hs Sultan cells, respectively. These data are summarized in FIG. 1.

### 6.3 Toxicology Studies

The effects of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) on cardiovascular and respiratory function are investigated in anesthetized dogs. Two groups of Beagle dogs (2/sex/group) are used. One group receives three doses of vehicle only and the other receives three ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (2, 10, and 20 mg/kg). In all cases, doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione or vehicle are successively administered via infusion through the jugular vein separated by intervals of at least 30 minutes.

The cardiovascular and respiratory changes induced by 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione are minimal at all doses when compared to the vehicle control group. The only statistically significant difference between the vehicle and treatment groups is a small increase in arterial blood pressure (from 94 mmHg to 101



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mmHg) following administration of the low dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. This effect lasts approximately 15 minutes and is not seen at higher doses. Deviations in femoral blood flow, respiratory parameters, and Qtc interval are common to both the control and treated groups and are not considered treatment-related.

#### 6.4 Cycling Therapy in Patients

In a specific embodiment, an immunomodulatory compound of the invention are cyclically administered to patients with cancer. Cycling therapy involves the administration of a first agent for a period of time, followed by a rest for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

In a specific embodiment, prophylactic or therapeutic agents are administered in a cycle of about 4 to 6 weeks, about once or twice every day. One cycle can comprise the administration of a therapeutic or prophylactic agent for three to four weeks and at least a week or two weeks of rest. The number of cycles administered is from about one to about 24 cycles, more typically from about two to about 16 cycles, and more typically from about four to about eight cycles.

For example, in a cycle of four weeks, on day 1, the administration of 25 mg/d of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is started. On day 22, the administration of the compound is stopped for a week of rest. On day 29, the administration of 25 mg/d 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is begun.

#### 6.5 Clinical Studies in Patients

##### 6.5.1 Treatment of Relapsed Multiple Myeloma

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) was administered to patients with relapsed/refractory multiple myeloma. The study was conducted in compliance with Good Clinical Practices. Patients were at least 18 years old, had been diagnosed with multiple myeloma (with paraprotein in serum and/or urine), and were considered refractory to treatment after at least two cycles of treatment, or have relapsed after two cycles of treatment.

Patients who have progressive disease, according to the Southwest Oncology Group (SWOG) criteria, on their prior regimen are considered treatment refractory. Relapse following remission is defined as >25% increase in M component from baseline levels; reappearance of the M paraprotein that had previously disappeared; or a definite increase in the size and number of lytic bone lesions recognized on radiographs. Patients may have had prior therapy with thalidomide, provided they were able to tolerate the treatment. A Zubrod performance status of 0 to 2 is required for all patients.

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione is administered to patients at doses of 1, 2, 5, or 10 mg/day for up to four weeks; at each dose level, three patients are initially enrolled. Dosing occurs at approximately the same time each morning; all doses are administered in the fasted state (no eating for at least two hours prior to dosing and two hours after dosing). 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione doses are administered in an ascending fashion such that patients in the first cohort receive the lowest dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (1 mg/day) and escalation to the next higher dose level occurs only following the establishment of safety and tolerability at the current dose. If one out of three patients at any dose level experience dose limiting toxicity (DLT), three additional patients are enrolled at that dose. If none of the three additional patients experience DLT, escalation to the next dose level occurs; dose escalations continue in a similar

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fashion until the MTD is established or the maximum daily dose (10 mg/day) is attained. However, if one of the three additional patients enrolled experiences DLT, the MTD has been reached. If two or more of the three additional patients enrolled experience DLT, the MTD is judged to have been exceeded and three additional patients are enrolled at the preceding dose level to confirm the MTD. Once the MTD has been identified, four additional patients are enrolled at that dose level so that a total of 10 patients is treated at the MTD.

Blood sampling for analysis of pharmacokinetic parameters is performed on Days 1 and 28 according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. An additional blood sample is collected at each weekly visit for the determination of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione levels. Total urine collections are also made with urine pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Safety assessments are made by monitoring adverse events, vital signs, ECGs, clinical laboratory evaluations (blood chemistry, hematology, lymphocyte phenotyping, and urinalysis), and physical examination at specific times during the study.

Results of interim pharmacokinetic analyses obtained following single- and multiple-dose administration of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione to multiple myeloma patients are presented below in Tables 1 and 2. These data show that 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione was steadily absorbed at all dose levels in relapsed multiple myeloma patients. Maximum plasma concentrations occurred at a median  $T_{max}$  of between 2.5 and 2.8 hours post-dose at Day 1 and between 3 and 4 hours post-dose at Week 4. At all doses, plasma concentrations declined in a monophasic manner after reaching  $C_{max}$ . The start of the elimination phase occurred between 3 and 10 hours post-dose at Day 1 and Week 4, respectively.

These data also showed that after 4 weeks of dosing, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione accumulated to a small extent (mean accumulation ratios ~1.02 to 1.52 and ~0.94 to 1.62 for  $C_{max}$  and  $AUC_{(0-\infty)}$ , respectively). There was almost a dose proportional increase in  $AUC_{(0-\infty)}$  and  $C_{max}$  values with increasing dose. A five-fold higher dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione produced a 3.2- and 2.2-fold increase in  $C_{max}$  at Day 1 and Week 4, respectively. Similarly, a 5-fold increase in dose resulted in a 3.6- and 2.3-fold increase in  $AUC_{(0-\infty)}$ , at Day 1 and Week 4, respectively.

TABLE 1

Pharmacokinetic parameters of Actimid™ in relapsed multiple myeloma patients				
Parameter		1 mg (N = 6)	2 mg (N = 2)	5 mg (N = 3)
		Day 1		
$C_{max}$	ng/mL	15.03 (4.04)	24.4* (12.1)	48.56 (14.03)
$t_{max}$	h	3.3 (2.6)	2.7* (0.3)	2.3 (0.3)
$AUC_{(0-\infty)}$	ng · h/mL	152.90 (36.62)	279.18 (51.10)	593.10 (335.23)
$AUC_{(0-\tau)}$		134.21 (27.14)	249.57 (29.26)	520.94 (267.32)
$t_{1/2}$	h	7.3 (3.4)	6.3 (1.4)	6.5 (2.2)
CL/F	mL/min	114.75 (29.20)	121.43 (22.22)	182.31 (117.06)
Vz/f	L	69.55 (44.97)	65.31 (2.80)	87.24 (22.61)

t = 24 hours

N/A = not available



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TABLE 2

Pharmacokinetic parameters of Actimid™ following multiple oral doses (1, 2, and 5 mg/day) in relapsed multiple myeloma patients				
Parameter	1 mg (N = 5)	2 mg (N = 2)	5 mg (N = 3)	
Week 4				
$C_{max}$	ng/mL	23.20 (7.48)	30.05* (15.64)	58.07 (38.08)
$t_{max}$	h	3.6 (1.5)	2.8* (0.3)	5.0 (2.6)
$AUC_{(0-\infty)}$	ng · h/mL	N/A	N/A	N/A
$AUC_{(0-\tau)}$		239.31 (122.59)	269.36 (186.34)	597.24 (354.23)
$t_{1/2}$	h	6.2* (0.6)	7.7 (2.8)	7.8 (4.0)
CL/F	mL/min	87.85 (48.48)	162.68 (112.54)	207.50 (175.41)
Vz/f	L	41.35* (8.84)	95.04 (35.39)	103.95 (27.25)

 $\tau$  = 24 hours

N/A = not available

\*N = 3 patients

### 6.5.2 Treatment of Relapsed Multiple Myeloma

Two Phase 1 clinical studies of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) have been conducted to identify the maximum tolerated dose (MTD) in patients with refractory or relapsed multiple myeloma. These studies have also characterized the safety profile of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione when ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione were given orally for up to 4 weeks. Patients started 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment at 5 mg/day with subsequent escalation to 10, 25, and 50 mg/day. Patients were enrolled for 28 days at their assigned dose, with the option of extended treatment for those who did not exhibit disease progression or experience dose limiting toxicity (DLT). Patients were evaluated for adverse events at each visit and the severity of these events was graded according to the National Cancer Institute (NCI) Common Toxicity Criteria. Patients were discontinued if they experienced DLT (Grade 3 or greater non-hematological, or Grade 4 hematological toxicity).

In this study, 27 patients were enrolled. All patients had relapsed multiple myeloma and 18 (72%) were refractory to salvage therapy. Among these patients, 15 had undergone prior autologous stem cell transplantation and 16 patients had received prior thalidomide treatment. The median number of prior regimens was 3 (range 2 to 6).

Blood and urine samples were collected for analysis of pharmacokinetic parameters on Days 1 and 28. Blood samples were collected according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. In addition, a blood sample was collected at each weekly clinic visit for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione determination. Total urine was collected and pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Response to treatment was assessed by M-protein quantification (by immunoelectrophoresis) from serum and a 24-hour urine collection, with creatinine clearance and 24-hour protein calculations undertaken at screening, baseline, Weeks 2 and 4, and monthly thereafter (or upon early termination). Bone marrow aspirations and/or tissue biopsy are also performed at Months 3, 6 and 12 if a patient's paraprotein serum concentration or 24-hour urine protein excretion declined to the next lower level, based on best response criteria. Preliminary results for the 28-day treatment period are summarized below.

Preliminary pharmacokinetic analyses based on these two studies indicated that AUC and  $C_{max}$  values increase proportionally with dose following single and multiple doses in

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multiple myeloma patients (as was seen in healthy volunteers). Further, there was no evidence of accumulation with multiple dosing as single dose  $AUC_{(0-\infty)}$  was comparable to multiple dose  $AUC_{(0-\tau)}$  following the same dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Similar to healthy volunteer studies, double peaks were observed. Exposure in multiple myeloma patients appeared to be slightly higher based on  $C_{max}$  and AUC values as compared to healthy male volunteers while clearance in multiple myeloma patients was lower than it was in healthy volunteers, consistent with their poorer renal function (both as a consequence of their age and their disease). Finally, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione half-life in patients was shorter than in healthy volunteers (mean 8 hours, ranging up to 17 hours).

In this study, the first cohort of 3 patients was treated for 28 days at 5 mg/day without any dose limiting toxicity (DLT). The second cohort of 3 patients subsequently commenced therapy at 10 mg/day. Patients in the second 10 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione cohort tolerated treatment well.

### 6.5.3 Treatment of Solid Tumors

Study with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) was conducted in patients with varying types of solid tumors, including malignant melanoma (13), carcinoma of the pancreas (2), carcinoid-unknown primary (1), renal carcinoma (1), breast carcinoma (1) and NSCLC (2). Patients received 5 mg/day 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for seven days and are subsequently escalated every seven days to 10 mg/day, 25 mg/day, and 50 mg/day for a total of 4 weeks of treatment. Patients who, experienced clinical benefit were permitted to continue on treatment as Named Patients.

The study initially enrolled 20 patients and was subsequently amended to enroll 16 additional patients (adrenal carcinoma, NSCLC, malignant mesothelioma, breast cancer, malignant melanoma (8), renal cell cancer (4)) at a higher dose. The 16 additional patients were given weekly escalating doses of 25 mg/day, 50 mg/day, 75 mg/day, 100 mg/day, 125 mg/day, and 150 mg/day over a 6-week period with continuing treatment for an additional six weeks.

The study of Phase 1 study was designed to determine a maximum tolerated dose (MTD) of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in patients with refractory solid tumors and/or lymphoma, as well as to characterize the pharmacokinetic and side effect profiles of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in this patient population. The study design dictates that at least 3 patients must be enrolled at a dose level and have completed 28 days of treatment prior to enrollment of patients at the next higher dose level. Patients in the first cohort began dosing at 5 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Patients will be escalated to 10, 20, 25, and 30 mg/day provided there is no toxicity.

In this study, the MTD is defined as the highest dose level in which fewer than two of six patients treated did not experience Grade 3 or greater non-hematological toxicity or Grade 4 or greater hematological toxicity. If, at any given dose level in either study, one out of three patients experiences toxicity, three additional patients must be treated at that particular dose. If, however, two out of six patients experience DLT, the MTD is judged to have been exceeded. No further dose escalations are to occur and additional patients are to be enrolled at the previous dose level. The dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered is escalated until the MTD is achieved or the maximum daily dose of is reached.

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No DLTs were reported in the initial group of 20 patients enrolled in the study. Thirteen of the original 20 trial patients, along with 2 non-trial patients, continued on treatment as named patients at doses up to 150 mg/day.

#### 6.5.4 Treatment of Gliomas

This study was performed to find toxicity in patients with recurrent, high-grade gliomas. The study is designed such that patients are given increasingly higher doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione until a maximum tolerated dose (MTD) is established. The study also seeks to obtain preliminary toxicity information and pharmacokinetic data on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, as well as to develop exploratory data concerning surrogate end points of angiogenic activity in vivo using functional neuro-imaging studies, and in vitro assays of serum angiogenic peptides.

Patients enrolled in the first cohort receive 2.5 mg/m<sup>2</sup>/day for a 4-week cycle. During each 4-week cycle of therapy, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered once daily for 3 weeks followed by a week of rest. Patients who complete a treatment cycle may receive another cycle of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment if two criteria are met. First, the patient must have stable disease or have experienced a partial response or complete response, or the patient is benefiting from the therapy with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione as evidenced by a decrease in tumor-related symptoms such as neurological deficits. Second, the patient must have recovered from toxicity related to 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione which occurred in the prior cycle by Day 42 or sooner (28-day cycle plus limit of 2 weeks to recover) as evidenced by a return to Grade  $\leq 1$  toxicity level. Patients who experience DLT in the previous cycle should have their dose modified. DLT is defined as a non-hematological event Grade  $\geq 3$  toxicity or hematological event of Grade 4 toxicity thought to be related to the study medication. Patients who experience DLT in the first cycle and have no response to therapy are removed from the study.

3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione doses are subsequently escalated to 5, 8, 11, 15, and 20 mg/m<sup>2</sup>/day to a maximum total daily dose of 40 mg. Patients continue to receive 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione on a 4-week cycle per dose level until one of the off-study criteria are met.

Three patients are enrolled in each cohort. If at least one DLT occurs, three additional patients are added to the cohort at that particular dose level. If two DLTs occur, the MTD, defined as the dose at which fewer than one-third of patients at each dose level experiences DLT has been exceeded and four more patients are treated at the previous dose.

Patients who experience DLT during the first 4-week cycle are removed from the study, except if they have a response to therapy. For patients who have completed their first 4-week cycle of without DLT, but who subsequently experience Grade 3 or 4 hematological and/or nonhematological toxicity, treatment is suspended for a minimum of a week. If the toxicity resolves to  $<$ Grade 2 within three weeks, the patient is treated at two dose levels lower than the dose that caused the toxicity (or a 50% reduction if the patient was treated at the first or second dose level). Patients in whom Grade 3 or 4 toxicity does not resolve to  $<$ Grade 1 within three weeks, or those who have another Grade 3 toxicity at the reduced dose are removed from the study.

Pharmacokinetic sampling is performed prior the first dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Day 1) and 0.5, 1, 2, 4, 6, 8, 24, and 48 hours

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thereafter. Sampling is also conducted pre-dose on Days 7 and 21 and 0.5, 1, 2, 4, 6, 8, and 24 post-dose on Day 21 to evaluate steady-state 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione levels.

#### 6.5.5 Treatment of Metastatic Melanoma

Patients with metastatic melanoma were started on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revmid™) at 5 mg/day for seven days. The dose was then increased every seven days to 10 mg/day, 25 mg/day, and 50 mg/day, respectively, for a total of four weeks on therapy. Five of the 13 melanoma patients who were treated under this regimen either showed disease stabilization or a partial response in the first four weeks of treatment. Tumor response was seen in cutaneous and subcutaneous lesions (five patients), lymph nodes (two patients), and liver (one patient). The duration of response was approximately six months. The result suggests that the compound appears is a promising new anti-cancer agent and has both antiangiogenic and immunomodulatory properties.

#### 6.5.6 Treatment of Relapsed or Refractory Multiple Myeloma

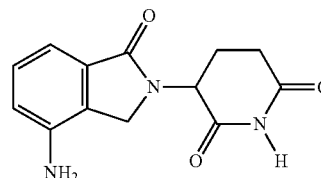
Patients with relapsed and refractory Dune-Salmon stage III multiple myeloma, who have either failed at least three previous regimens or presented with poor performance status, neutropenia or thrombocytopenia, are treated with up to four cycles of combination of melphalan (50 mg intravenously), an immunomodulatory compound of the invention (about 1 to 150 mg orally daily), and dexamethasone (40 mg/day orally on days 1 to 4) every four to six weeks. Maintenance treatment consisting of daily an immunomodulatory compound of the invention and monthly dexamethasone are continued until the disease progression. The therapy using an immunomodulatory compound of the invention in combination with melphalan and dexamethasone is highly active and generally tolerated in heavily pretreated multiple myeloma patients whose prognosis is otherwise poor.

The embodiments of the invention described above are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials, and procedures. All such equivalents are considered to be within the scope of the invention and are encompassed by the appended claims.

What is claimed is:

1. A method of treating multiple myeloma, which comprises administering, on a 28 day cycle, to a patient having multiple myeloma:

(a) about 25 mg per day of a compound of the formula:



or a pharmaceutically acceptable salt or solvate thereof, for 21 consecutive days followed by seven consecutive days of rest from administration of the compound, and;

(b) 40 mg per day of dexamethasone on days 1 through 4 in the 28 day cycle, wherein the patient has received previous therapy for multiple myeloma.

2. The method of claim 1, wherein the multiple myeloma is relapsed multiple myeloma.

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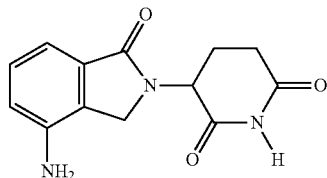
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3. The method of claim 1, wherein the multiple myeloma is refractory multiple myeloma.

4. The method of claim 1, wherein the multiple myeloma is relapsed and refractory multiple myeloma.

5. The method of claim 1, wherein the patient has demonstrated disease progression on the previous therapy.

6. The method of claim 1, wherein the compound is



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and is not a pharmaceutically acceptable salt or solvate thereof.

7. The method of claim 1, wherein the compound is a pharmaceutically acceptable salt.

8. The method of claim 1, wherein the compound is a pharmaceutically acceptable solvate.

9. The method of claim 1, wherein the compound is administered orally.

10. The method of claim 1, wherein the compound is administered in the form of a capsule.

11. The method of claim 1, wherein the compound is administered in the form of a tablet.

12. The method of claim 10, wherein the capsule comprises the compound, lactose anhydrous, microcrystalline cellulose, croscarmellose sodium and magnesium stearate.

13. The method of claim 1, wherein the dexamethasone is administered orally.

\* \* \* \* \*

# **EXHIBIT G**



US008648095B2

(12) **United States Patent**  
**Zeldis**

(10) **Patent No.:** **US 8,648,095 B2**  
(45) **Date of Patent:** **\*Feb. 11, 2014**

(54) **METHODS FOR TREATING MULTIPLE MYELOMA USING 3-(4-AMINO-1-OXO-1,3-DIHYDROISOINDOL-2-YL)-PIPERIDINE-2,6-DIONE IN COMBINATION WITH PROTEASOME INHIBITOR**

(75) Inventor: **Jerome B. Zeldis**, Princeton, NJ (US)

(73) Assignee: **Celgene Corporation**, Summit, NJ (US)

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.  
This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/488,888**

(22) Filed: **Jun. 5, 2012**

(65) **Prior Publication Data**

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(51) **Int. Cl.**  
**A01N 43/40** (2006.01)

(52) **U.S. Cl.**  
USPC ..... **514/321**; 514/323

(58) **Field of Classification Search**  
USPC ..... 514/323  
See application file for complete search history.

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(57) **ABSTRACT**

Methods of treating, preventing and/or managing cancer as well as and diseases and disorders associated with, or characterized by, undesired angiogenesis are disclosed. Specific methods encompass the administration of an immunomodulatory compound alone or in combination with a second active ingredient. The invention further relates to methods of reducing or avoiding adverse side effects associated with chemotherapy, radiation therapy, hormonal therapy, biological therapy or immunotherapy which comprise the administration of an immunomodulatory compound. Pharmaceutical compositions, single unit dosage forms, and kits suitable for use in methods of the invention are also disclosed.

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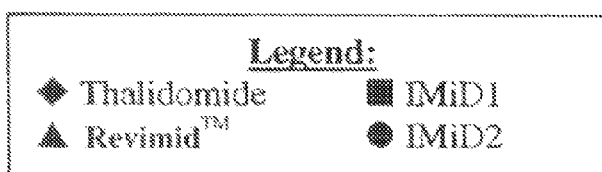
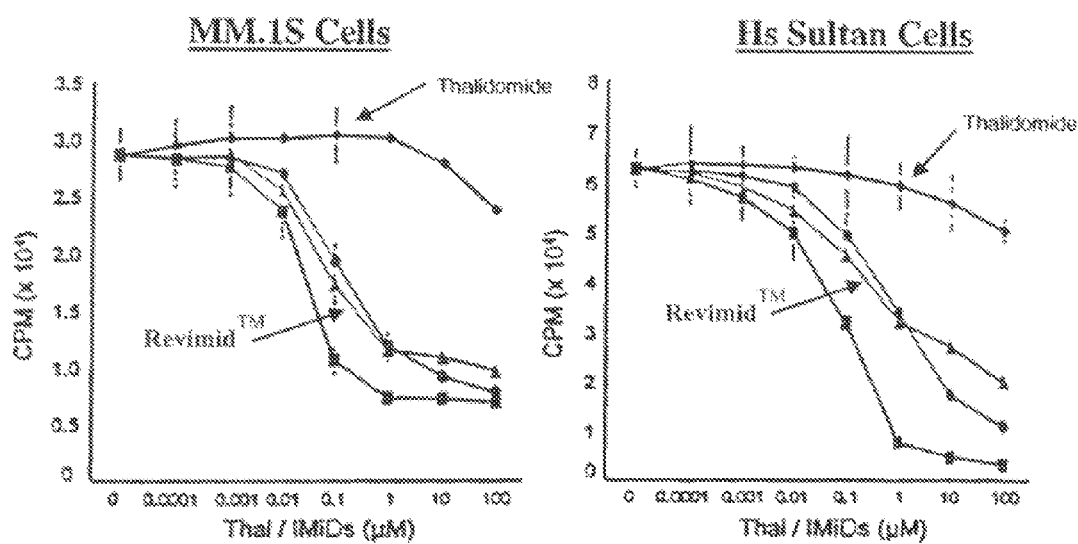
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**U.S. Patent****Feb. 11, 2014****US 8,648,095 B2****Effects of Revimid™ and Thalidomide on MM Cell Proliferation**

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**METHODS FOR TREATING MULTIPLE  
MYELOMA USING 3-(4-AMINO-1-OXO-1,3-  
DIHYDROISOINDOL-2-YL)-PIPERIDINE-2,6-  
DIONE IN COMBINATION WITH  
PROTEASOME INHIBITOR**

This application is a continuation of U.S. patent application Ser. No. 12/640,702, filed Dec. 17, 2009, now U.S. Pat. No. 8,198,306, which is a continuation application of U.S. patent application Ser. No. 10/438,213, filed May 15, 2003, now U.S. Pat. No. 7,968,569, issued Jun. 28, 2011, which claims the benefit of U.S. provisional application Nos. 60/380,842, filed May 17, 2002, and 60/424,600, filed Nov. 6, 2002, the entireties of which are incorporated herein by reference.

## 1. FIELD OF THE INVENTION

This invention relates to methods of treating, preventing and/or managing specific cancers, and other diseases including, but not limited to, those associated with, or characterized by, undesired angiogenesis, by the administration of one or more immunomodulatory compounds alone or in combination with other therapeutics. In particular, the invention encompasses the use of specific combinations, or "cocktails," of drugs and other therapy, e.g., radiation to treat these specific cancers, including those refractory to conventional therapy. The invention also relates to pharmaceutical compositions and dosing regimens.

## 2. BACKGROUND OF THE INVENTION

### 2.1 Pathobiology of Cancer and Other Diseases

Cancer is characterized primarily by an increase in the number of abnormal cells derived from a given normal tissue, invasion of adjacent tissues by these abnormal cells, or lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites (metastasis). Clinical data and molecular biologic studies indicate that cancer is a multistep process that begins with minor preneoplastic changes, which may under certain conditions progress to neoplasia. The neoplastic lesion may evolve clonally and develop an increasing capacity for invasion, growth, metastasis, and heterogeneity, especially under conditions in which the neoplastic cells escape the host's immune surveillance. Roitt, I., Brostoff, J and Kale, D., *Immunology*, 17.1-17.12 (3rd ed., Mosby, St. Louis, Mo., 1993).

There is an enormous variety of cancers which are described in detail in the medical literature. Examples includes cancer of the lung, colon, rectum, prostate, breast, brain, and intestine. The incidence of cancer continues to climb as the general population ages, as new cancers develop, and as susceptible populations (e.g., people infected with AIDS or excessively exposed to sunlight) grow. A tremendous demand therefore exists for new methods and compositions that can be used to treat patients with cancer.

Many types of cancers are associated with new blood vessel formation, a process known as angiogenesis. Several of the mechanisms involved in tumor-induced angiogenesis have been elucidated. The most direct of these mechanisms is the secretion by the tumor cells of cytokines with angiogenic properties. Examples of these cytokines include acidic and basic fibroblastic growth factor (a,b-FGF), angiogenin, vascular endothelial growth factor (VEGF), and TNF- $\alpha$ . Alternatively, tumor cells can release angiogenic peptides through the production of proteases and the subsequent breakdown of the extracellular matrix where some cytokines are stored

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(e.g., b-FGF). Angiogenesis can also be induced indirectly through the recruitment of inflammatory cells (particularly macrophages) and their subsequent release of angiogenic cytokines (e.g., TNF- $\alpha$ , bFGF).

A variety of other diseases and disorders are also associated with, or characterized by, undesired angiogenesis. For example, enhanced or unregulated angiogenesis has been implicated in a number of diseases and medical conditions including, but not limited to, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, rubeosis (neovascularization of the angle), viral diseases, genetic diseases, inflammatory diseases, allergic diseases, and autoimmune diseases. Examples of such diseases and conditions include, but are not limited to: diabetic retinopathy; retinopathy of prematurity; corneal graft rejection; neovascular glaucoma; retrolental fibroplasia; and proliferative vitreoretinopathy.

Accordingly, compounds that can control angiogenesis or inhibit the production of certain cytokines, including TNF- $\alpha$ , may be useful in the treatment and prevention of various diseases and conditions.

### 2.2 Methods of Treating Cancer

Current cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient (see, for example, Stockdale, 1998, *Medicine*, vol. 3, Rubenstein and Federman, eds., Chapter 12, Section IV). Recently, cancer therapy could also involve biological therapy or immunotherapy. All of these approaches pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of a patient or may be unacceptable to the patient. Additionally, surgery may not completely remove neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue. Radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent. Although hormonal therapy can be effective, it is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of cancer cells. Biological therapies and immunotherapies are limited in number and may produce side effects such as rashes or swellings, flu-like symptoms, including fever, chills and fatigue, digestive tract problems or allergic reactions.

With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A majority of cancer chemotherapeutics act by inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division. Gilman et al., *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, Tenth Ed. (McGraw Hill, New York).

Despite availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks. Stockdale, *Medicine*, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. 10, 1998. Almost all chemotherapeutic agents are toxic, and chemotherapy causes significant, and often dangerous side effects including severe nausea, bone marrow depression, and immunosuppression. Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even if those agents act by different mechanism from those of the drugs used in the specific treatment. This phenomenon is referred to as pleio-



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tropic drug or multidrug resistance. Because of the drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

Other diseases or conditions associated with, or characterized by, undesired angiogenesis are also difficult to treat. However, some compounds such as protamine, heparin and steroids have been proposed to be useful in the treatment of certain specific diseases. Taylor et al., *Nature* 297:307 (1982); Folkman et al., *Science* 221:719 (1983); and U.S. Pat. Nos. 5,001,116 and 4,994,443. Thalidomide and certain derivatives of it have also been proposed for the treatment of such diseases and conditions. U.S. Pat. Nos. 5,593,990, 5,629,327, 5,712,291, 6,071,948 and 6,114,355 to D'Amato.

Still, there is a significant need for safe and effective methods of treating, preventing and managing cancer and other diseases and conditions, particularly for diseases that are refractory to standard treatments, such as surgery, radiation therapy, chemotherapy and hormonal therapy, while reducing or avoiding the toxicities and/or side effects associated with the conventional therapies.

### 2.3 IMiDs™

A number of studies have been conducted with the aim of providing compounds that can safely and effectively be used to treat diseases associated with abnormal production of TNF- $\alpha$ . See, e.g., Marriott, J. B., et al., *Expert Opin. Biol. Ther.* 1(4):1-8 (2001); G. W. Muller, et al., *Journal of Medicinal Chemistry* 39(17): 3238-3240 (1996); and G. W. Muller, et al., *Bioorganic & Medicinal Chemistry Letters* 8: 2669-2674 (1998). Some studies have focused on a group of compounds selected for their capacity to potentially inhibit TNF- $\alpha$  production by LPS stimulated PBMC. L. G. Corral, et al., *Ann. Rheum. Dis.* 58(Suppl 1) 1107-1113 (1999). These compounds, which are referred to as IMiDs™ (Celgene Corporation) or Immunomodulatory Drugs, show not only potent inhibition of TNF- $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  and IL12 production. LPS induced IL6 is also inhibited by immunomodulatory compounds, albeit partially. These compounds are potent stimulators of LPS induced IL10. Id. Particular examples of IMiDs™ include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles described in U.S. Pat. Nos. 6,281,230 and 6,316,471, both to G. W. Muller, et al.

### 3. SUMMARY OF THE INVENTION

This invention encompasses methods of treating and preventing certain types of cancer, including primary and metastatic cancer, as well as cancers that are refractory or resistant to conventional chemotherapy. The methods comprise administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. The invention also encompasses methods of managing certain cancers (e.g., preventing or prolonging their recurrence, or lengthening the time of remission) which comprise administering to a patient in need of such management a prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage cancer. Examples of such conventional therapies include, but are

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not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention also encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are associated with, or characterized by, undesired angiogenesis, which comprise administering to a patient in need of such treatment, management or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In other methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage diseases or disorders associated with, or characterized by, undesired angiogenesis. Examples of such conventional therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention encompasses pharmaceutical compositions, single unit dosage forms, dosing regimens and kits which comprise an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second, or additional, active agent. Second active agents include specific combinations, or "cocktails," of drugs.

### 4. BRIEF DESCRIPTION OF FIGURE

FIG. 1 shows a comparison of the effects of 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide in inhibiting the proliferation of multiple myeloma (MM) cell lines in an in vitro study. The uptake of [<sup>3</sup>H]-thymidine by different MM cell lines (MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of the cell proliferation.

### 5. DETAILED DESCRIPTION OF THE INVENTION

A first embodiment of the invention encompasses methods of treating, managing, or preventing cancer which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with another drug ("second active agent") or method of treating, managing, or preventing cancer. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage cancer.

Another embodiment of the invention encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are characterized by undesired angiogenesis. These methods comprise the administration of a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

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Examples of diseases and disorders associated with, or characterized by, undesired angiogenesis include, but are not limited to, inflammatory diseases, autoimmune diseases, viral diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, and rubeosis (neovascularization of the angle).

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with a second active agent or method of treating, managing, or preventing the disease or condition. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage disease and conditions associated with, or characterized by, undesired angiogenesis.

The invention also encompasses pharmaceutical compositions (e.g., single unit dosage forms) that can be used in methods disclosed herein. Particular pharmaceutical compositions comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second active agent.

#### 5.1 Immunomodulatory Compounds

Compounds used in the invention include immunomodulatory compounds that are racemic, stereomerically enriched or stereomerically pure, and pharmaceutically acceptable salts, solvates, hydrates, stereoisomers, clathrates, and prodrugs thereof. Preferred compounds used in the invention are small organic molecules having a molecular weight less than about 1,000 g/mol, and are not proteins, peptides, oligonucleotides, oligosaccharides or other macromolecules.

As used herein and unless otherwise indicated, the terms “immunomodulatory compounds” and “IMiDs™” (Celgene Corporation) encompasses small organic molecules that markedly inhibit TNF- $\alpha$ , LPS induced monocyte IL1 $\beta$  and IL12, and partially inhibit IL6 production. Specific immunomodulatory compounds are discussed below.

TNF- $\alpha$  is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation. TNF- $\alpha$  is responsible for a diverse range of signaling events within cells. TNF- $\alpha$  may play a pathological role in cancer. Without being limited by theory, one of the biological effects exerted by the immunomodulatory compounds of the invention is the reduction of synthesis of TNF- $\alpha$ . Immunomodulatory compounds of the invention enhance the degradation of TNF- $\alpha$  mRNA.

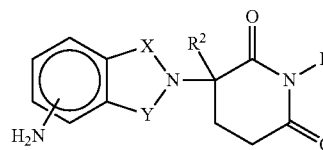
Further, without being limited by theory, immunomodulatory compounds used in the invention may also be potent co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. Immunomodulatory compounds of the invention may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset. In addition, the compounds preferably have anti-inflammatory properties, and efficiently co-stimulate T cells.

Specific examples of immunomodulatory compounds of the invention, include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those dis-

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closed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl)isoindolines such as those described in U.S. Pat. No. 5,874,448; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolines described in U.S. Pat. No. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines (e.g., 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517; and a class of non-polypeptide cyclic amides disclosed in U.S. Pat. Nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S. Pat. Nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles such as those described in U.S. Pat. Nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those described in U.S. patent application Ser. No. 09/972,487 filed on Oct. 5, 2001, U.S. patent application Ser. No. 10/032,286 filed on Dec. 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents and patent applications identified herein are incorporated herein by reference. Immunomodulatory compounds of the invention do not include thalidomide.

Other specific immunomodulatory compounds of the invention include, but are not limited to, 1-oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein by reference. These compounds have the structure I:



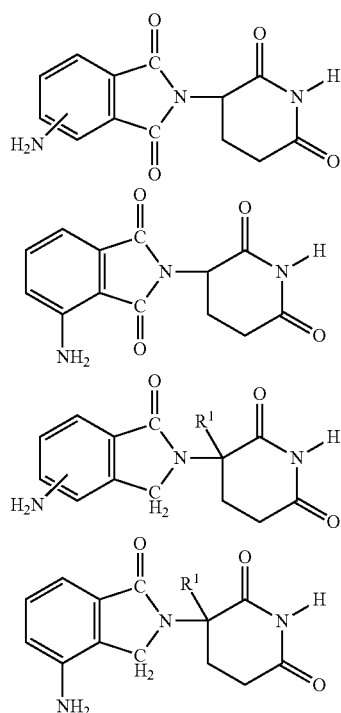
in which one of X and Y is C=O, the other of X and Y is C=O or CH<sub>2</sub>, and R<sup>2</sup> is hydrogen or lower alkyl, in particular methyl. Specific immunomodulatory compounds include, but are not limited to:

1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;  
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
and  
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

Other specific immunomodulatory compounds of the invention belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles, such as those described in U.S. Pat. Nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (International Publication No. WO 98/03502), each of which is incorporated herein by reference. Compounds representative of this class are of the formulas:

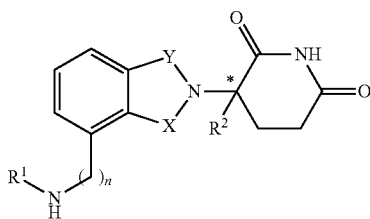
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wherein  $R^1$  is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (e.g. optically pure (R) or (S) enantiomers) of these compounds.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. Nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are incorporated herein by reference. Representative compounds are of formula II:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C≡O;

$R^1$  is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, C(O)R<sup>3</sup>, C(S)R<sup>3</sup>, C(O)OR<sup>4</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, C(O)NHR<sup>3</sup>, C(S)NHR<sup>3</sup>, C(O)NR<sup>3</sup>R<sup>3'</sup>, C(S)NR<sup>3</sup>R<sup>3'</sup> or (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>;

$R^2$  is H, F, benzyl, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, or (C<sub>2</sub>-C<sub>8</sub>)alkynyl;

$R^3$  and  $R^{3'}$  are independently (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-

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C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>0</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>, or C(O)OR<sup>5</sup>;

$R^4$  is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, (C<sub>1</sub>-C<sub>4</sub>)alkyl-OR<sup>5</sup>, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, or (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl;

$R^5$  is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, or (C<sub>2</sub>-C<sub>5</sub>)heteroaryl;

each occurrence of  $R^6$  is independently H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>2</sub>-C<sub>5</sub>)heteroaryl, or (C<sub>0</sub>-C<sub>8</sub>)alkyl-C(O)O—R<sup>5</sup> or the  $R^6$  groups can join to form a heterocycloalkyl group;

n is 0 or 1; and

\* represents a chiral-carbon center.

In specific compounds of formula II, when n is 0 then  $R^1$  is (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, C(O)R<sup>3</sup>, C(O)OR<sup>4</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, C(S)NHR<sup>3</sup>, or (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>;

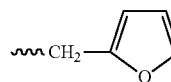
$R^2$  is H or (C<sub>1</sub>-C<sub>8</sub>)alkyl; and

$R^3$  is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>5</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>; (C<sub>0</sub>-C<sub>8</sub>)alkyl-NH—C(O)O—R<sup>5</sup>; (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>, or C(O)OR<sup>5</sup>; and the other variables have the same definitions.

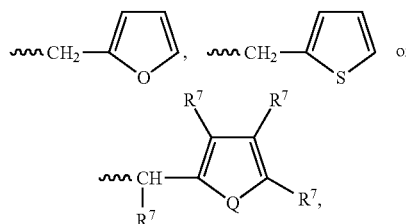
In other specific compounds of formula II,  $R^2$  is H or (C<sub>1</sub>-C<sub>4</sub>)alkyl.

In other specific compounds of formula II,  $R^1$  is (C<sub>1</sub>-C<sub>8</sub>)alkyl or benzyl.

In other specific compounds of formula II,  $R^1$  is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, benzyl, CH<sub>2</sub>OCH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, or



In another embodiment of the compounds of formula II,  $R^1$  is



wherein Q is O or S, and each occurrence of  $R^7$  is independently H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, benzyl, CH<sub>2</sub>OCH<sub>3</sub>, or CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>.

In other specific compounds of formula II,  $R^1$  is C(O)R<sup>3</sup>.

In other specific compounds of formula II,  $R^3$  is (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>1</sub>-C<sub>8</sub>)alkyl, aryl, or (C<sub>0</sub>-C<sub>4</sub>)alkyl-OR<sup>5</sup>.

In other specific compounds of formula II, heteroaryl is pyridyl, furyl, or thienyl.

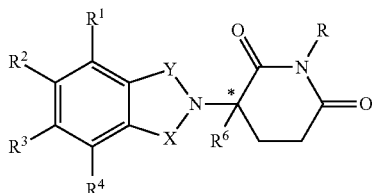
In other specific compounds of formula II,  $R^1$  is C(O)OR<sup>4</sup>.

In other specific compounds of formula II, the H of C(O)NHC(O) can be replaced with (C<sub>1</sub>-C<sub>4</sub>)alkyl, aryl, or benzyl.

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Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. No. 09/781,179, International Publication No. WO 98/54170, and U.S. Pat. No. 6,395,754, each of which are incorporated herein by reference. Representative compounds are of formula III:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

R is H or  $\text{CH}_2\text{OCOR}'$ :

(i) each of  $R^1$ ,  $R^2$ ,  $R^3$ , or  $R^4$ , independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of  $R^1$ ,  $R^2$ ,  $R^3$ , or  $R^4$  is nitro or  $-NHR^5$  and the remaining of  $R^1$ ,  $R^2$ ,  $R^3$ , or  $R^4$  are hydrogen;

R<sup>5</sup> is hydrogen or alkyl of 1 to 8 carbons

R<sup>6</sup> hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro;

$$R' \text{ is } R^7\text{---CHR}^{10}\text{---N}(R^8R^9);$$

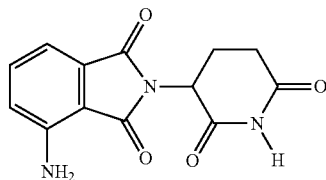
R<sup>7</sup> is m-phenylene or p-phenylene or  $-(C_n H_{2n})-$  in which n has a value of 0 to 4;

each of R<sup>8</sup> and R<sup>9</sup> taken independently of the other is hydrogen or alkyl of 1 to 8 carbon atoms, or R<sup>8</sup> and R<sup>9</sup> taken together are tetramethylene, pentamethylene, hexamethylene, or —CH<sub>2</sub>CH<sub>2</sub>[X]X<sub>1</sub>CH<sub>2</sub>CH<sub>2</sub>— in which [X]X<sub>1</sub> is —O—, —S—, or —NH—;

R<sup>10</sup> is hydrogen, alkyl of to 8 carbon atoms, or phenyl; and

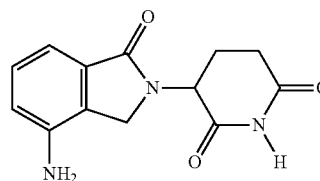
\* represents a chiral-carbon center.

The most preferred immunomodulatory compounds of the invention are 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. The compounds can be obtained via standard, synthetic methods (see e.g., U.S. Pat. No. 5,635, 517, incorporated herein by reference). The compounds are available from Celgene Corporation, Warren, N.J. 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (ACTIMID™) has the following chemical structure:



The compound 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (REVIMID™) has the following chemical structure:

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Compounds of the invention can either be commercially purchased or prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry techniques.

As used herein and unless otherwise indicated, the term “pharmaceutically acceptable salt” encompasses non-toxic acid and base addition salts of the compound to which the term refers. Acceptable non-toxic acid addition salts include those derived from organic and inorganic acids or bases known in the art, which include, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embolic acid, enanthic acid, and the like.

Compounds that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds are those that form non-toxic base addition salts, i.e., salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), lysine, and procaine.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of immunomodulatory compounds of the invention that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of immunomodulatory compounds of the invention that comprise —NO, —NO<sub>2</sub>, —ONO, or —ONO<sub>2</sub> moieties. Prodrugs can typically be prepared using well-known methods, such as those described in 1 *Burger's Medicinal Chemistry and Drug Discovery*, 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995), and *Design of Prodrugs* (H. Bundgaard ed., Elsevier, New York 1985).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties *in vivo*, such as uptake, duration of action, or onset of action; or 2) is biologi-



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cally inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxyethyl, acetoxyethyl, aminocarbonyloxyethyl, pivaloyloxyethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyl-oxyethyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides,  $\alpha$ -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, amino acids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

Various immunomodulatory compounds of the invention contain one or more chiral centers, and can exist as racemic mixtures of enantiomers or mixtures of diastereomers. This invention encompasses the use of stereomerically pure forms of such compounds, as well as the use of mixtures of those forms. For example, mixtures comprising equal or unequal amounts of the enantiomers of a particular immunomodulatory compounds of the invention may be used in methods and compositions of the invention. These isomers may be asymmetrically synthesized or resolved using standard techniques such as chiral columns or chiral resolving agents. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. As used herein and unless otherwise indicated, the term "stereomerically enriched" means a composition that comprises greater than about 60% by weight of one stereoisomer of a compound, preferably greater than about 70% by weight, more preferably greater than about 80% by weight of one stereoisomer of a compound. As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center. Similarly, the term "stereomerically enriched" means a stereomerically enriched composition of a compound having one chiral center.

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It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

## 5.2 Second Active Agents

Immunomodulatory compounds can be combined with other pharmacologically active compounds ("second active agents") in methods and compositions of the invention. It is believed that certain combinations work synergistically in the treatment of particular types of cancer and certain diseases and conditions associated with, or characterized by, undesired angiogenesis. Immunomodulatory compounds can also work to alleviate adverse effects associated with certain second active agents, and some second active agents can be used to alleviate adverse effects associated with immunomodulatory compounds.

One or more second active ingredients or agents can be used in the methods and compositions of the invention together with an immunomodulatory compound. Second active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules).

Examples of large molecule active agents include, but are not limited to, hematopoietic growth factors, cytokines, and monoclonal and polyclonal antibodies. Typical large molecule active agents are biological molecules, such as naturally occurring or artificially made proteins. Proteins that are particularly useful in this invention include proteins that stimulate the survival and/or proliferation of hematopoietic precursor cells and immunologically active poietic cells in vitro or in vivo. Others stimulate the division and differentiation of committed erythroid progenitors in cells in vitro or in vivo. Particular proteins include, but are not limited to: interleukins, such as IL-2 (including recombinant IL-II ("rIL2") and canarypox IL-2), IL-10, IL-12, and IL-18; interferons, such as interferon alfa-2a, interferon alfa-2b, interferon alfa-n1, interferon alfa-n3, interferon beta-1 a, and interferon gamma-I b; GM-CSF and GM-CSF; and EPO.

Particular proteins that can be used in the methods and compositions of the invention include, but are not limited to: filgrastim, which is sold in the United States under the trade name Neupogen® (Amgen, Thousand Oaks, Calif.); sargramostim, which is sold in the United States under the trade name Leukine® (Immunex, Seattle, Wash.); and recombinant EPO, which is sold in the United States under the trade name Epogen® (Amgen, Thousand Oaks, Calif.).

Recombinant and mutated forms of GM-CSF can be prepared as described in U.S. Pat. Nos. 5,391,485; 5,393,870; and 5,229,496; all of which are incorporated herein by reference. Recombinant and mutated forms of G-CSF can be prepared as described in U.S. Pat. Nos. 4,810,643; 4,999,291; 5,528,823; and 5,580,755; all of which are incorporated herein by reference.

This invention encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term "mutants" are proteins that lack carbohydrate moieties normally present in their naturally occurring forms

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(e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001).

Antibodies that can be used in combination with compounds of the invention include monoclonal and polyclonal antibodies. Examples of antibodies include, but are not limited to, trastuzumab (Herceptin®), rituximab (Rituxan®), bevacizumab (Avastin™), pertuzumab (Omnitarg™), tositumomab (Bexxar®), edrecolomab (Panorex®), and G250. Compounds of the invention can also be combined with, or used in combination with, anti-TNF- $\alpha$  antibodies.

Large molecule active agents may be administered in the form of anti-cancer vaccines. For example, vaccines that secrete, or cause the secretion of, cytokines such as IL-2, G-CSF, and GM-CSF can be used in the methods, pharmaceutical compositions, and kits of the invention. See, e.g., Emens, L. A., et al., *Curr. Opinion Mol. Ther.* 3(1):77-84 (2001).

In one embodiment of the invention, the large molecule active agent reduces, eliminates, or prevents an adverse effect associated with the administration of an immunomodulatory compound. Depending on the particular immunomodulatory compound and the disease or disorder being treated, adverse effects can include, but are not limited to, drowsiness and somnolence, dizziness and orthostatic hypotension, neutropenia, infections that result from neutropenia, increased HIV-viral load, bradycardia, Stevens-Johnson Syndrome and toxic epidermal necrolysis, and seizures (e.g., grand mal convulsions). A specific adverse effect is neutropenia.

Second active agents that are small molecules can also be used to alleviate adverse effects associated with the administration of an immunomodulatory compound. However, like some large molecules, many are believed to be capable of providing a synergistic effect when administered with (e.g., before, after or simultaneously) an immunomodulatory compound. Examples of small molecule second active agents include, but are not limited to, anti-cancer agents, antibiotics, immunosuppressive agents, and steroids.

Examples of anti-cancer agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; celecoxib (COX-2 inhibitor); chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epiropropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; iproplatin; irinotecan; irinotecan

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hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocil; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; pipsulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procabazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiro-mustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; taxotere; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; tretolone acetate; tricitribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; and zorubicin hydrochloride.

Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betacalamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; capecitabine; carboxamide-amino-triazole; carboxamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroquinoline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conage-nin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatin; cypemycin; cytarabine ocfosfate; cytolytic factor; cytosatin; dacliximab; decitabine; dehydrididemnin B; deslorelin; dexamethasone; dexifosfamide; dextrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; doxorubicin; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride;

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estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flzelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilomofosine; ilomastat; imatinib (e.g., Gleevec®), imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; Erbitux, human chorionic gonadotrophin; monophosphoryl lipid A+mycobacterium cell wall sk; mopidamol; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullin; oblimersen (Genasense®); O<sup>6</sup>-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiriomycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin;

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spongistatin 1; squalamine; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; tallimustine; tamoxifen methiodide; taumustine; tazartene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; velaresol; veramine; verdins; verteporfin; vinorelbine; vinoxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Specific second active agents include, but are not limited to, oblimersen (Genasense®), remicade, docetaxel, celecoxib, melphalan, dexamethasone (Decadron®), steroids, gemcitabine, cisplatin, temozolomide, etoposide, cyclophosphamide, temodar, carboplatin, procarbazine, gliadel, tamoxifen, topotecan, methotrexate, Arisa®, taxol, taxotere, fluorouracil, leucovorin, irinotecan, xeloda, CPT-11, interferon alpha, pegylated interferon alpha (e.g., PEG INTRON-A), capecitabine, cisplatin, thiotepa, fludarabine, carboplatin, liposomal daunorubicin, cytarabine, doxorubicin, paclitaxel, vinblastine, IL-2, GM-CSF, dacarbazine, vinorelbine, zoledronic acid, palmitronate, biaxin, busulphan, prednisone, bisphosphonate, arsenic trioxide, vincristine, doxorubicin (Doxil®), paclitaxel, ganciclovir, adriamycin, estramustine sodium phosphate (Emcyt®), sulindac, and etoposide.

### 5.3 Methods of Treatments and Prevention

Methods of this invention encompass methods of treating, preventing and/or managing various types of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis. As used herein, unless otherwise specified, the term “treating” refers to the administration of a compound of the invention or other additional active agent after the onset of symptoms of the particular disease or disorder. As used herein, unless otherwise specified, the term “preventing” refers to the administration prior to the onset of symptoms, particularly to patients at risk of cancer, and other diseases and disorders associated with, or characterized by, undesired angiogenesis. The term “prevention” includes the inhibition of a symptom of the particular disease or disorder. Patients with familial history of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis are preferred candidates for preventive regimens. As used herein and unless otherwise indicated, the term “managing” encompasses preventing the recurrence of the particular disease or disorder in a patient who had suffered from it, and/or lengthening the time a patient who had suffered from the disease or disorder remains in remission.

As used herein, the term “cancer” includes, but is not limited to, solid tumors and blood born tumors. The term “cancer” refers to disease of skin tissues, organs, blood, and vessels, including, but not limited to, cancers of the bladder, bone or blood, brain, breast, cervix, chest, colon, endometrium, esophagus, eye, head, kidney, liver, lymph nodes, lung, mouth, neck, ovaries, pancreas, prostate, rectum, stomach, testis, throat, and uterus. Specific cancers include, but are not limited to, advanced malignancy, amyloidosis, neuroblastoma, meningioma, hemangiopericytoma, multiple brain metastase, glioblastoma multiforms, glioblastoma, brain stem glioma, poor prognosis malignant brain tumor,



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malignant glioma, recurrent malignant glioma, anaplastic astrocytoma, anaplastic oligodendroglioma, neuroendocrine tumor, rectal adenocarcinoma, Dukes C & D colorectal cancer, unresectable colorectal carcinoma, metastatic hepatocellular carcinoma, Kaposi's sarcoma, karotype acute myeloblastic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma, low grade follicular lymphoma, malignant melanoma, malignant mesothelioma, malignant pleural effusion mesothelioma syndrome, peritoneal carcinoma, papillary serous carcinoma, gynecologic sarcoma, soft tissue sarcoma, scleroderma, cutaneous vasculitis, Langerhans cell histiocytosis, leiomyosarcoma, fibrodysplasia ossificans progressive, hormone refractory prostate cancer, resected high-risk soft tissue sarcoma, unresectable hepatocellular carcinoma, Waldenstrom's macroglobulinemia, smoldering myeloma, indolent myeloma, fallopian tube cancer, androgen independent prostate cancer, androgen dependent stage IV non-metastatic prostate cancer, hormone-insensitive prostate cancer, chemotherapy-insensitive prostate cancer, papillary thyroid carcinoma, follicular thyroid carcinoma, medullary thyroid carcinoma, and leiomyoma. In a specific embodiment, the cancer is metastatic. In another embodiment, the cancer is refractory or resistance to chemotherapy or radiation; in particular, refractory to thalidomide.

As used herein to refer to diseases and conditions other than cancer, the terms "diseases or disorders associated with, or characterized by, undesired angiogenesis," "diseases or disorders associated with undesired angiogenesis," and "diseases or disorders characterized by undesired angiogenesis" refer to diseases, disorders and conditions that are caused, mediated or attended by undesired, unwanted or uncontrolled angiogenesis, including, but not limited to, inflammatory diseases, autoimmune diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroidal neovascular diseases, and retina neovascular diseases.

Examples of such diseases or disorders associated with undesired angiogenesis include, but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, proliferative vitreoretinopathy, trachoma, myopia, optic pits, epidemic keratoconjunctivitis, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, syphilis, lipid degeneration, bacterial ulcer, fungal ulcer, Herpes simplex infection, Herpes zoster infection, protozoan infection, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, peripheral radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Behcet's disease, retinitis, choroiditis, presumed ocular histoplasmosis, Bests disease, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, rubeosis, sarcoidosis, sclerosis, soriatis, psoriasis, primary sclerosing cholangitis, proctitis, primary biliary srosis, idiopathic pulmonary fibrosis, and alcoholic hepatitis.

In specific embodiments of the invention, diseases or disorders associated with undesired angiogenesis do not include congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, myocardial infarction, HIV, hepatitis, adult respiratory distress syndrome, bone-resorption disease, chronic obstructive pulmonary diseases, chronic pul-

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monary inflammatory disease, dermatitis, cystic fibrosis, septic shock, sepsis, endotoxic shock, hemodynamic shock, sepsis syndrome, post ischemic reperfusion injury, meningitis, psoriasis, fibrotic disease, cachexia, graft rejection, rheumatoid spondylitis, osteoporosis, Crohn's disease, ulcerative colitis, inflammatory-bowel disease, multiple sclerosis, systemic lupus erythematosus, erythema nodosum leprosum in leprosy, radiation damage, asthma, hyperoxic alveolar injury, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

This invention encompasses methods of treating patients who have been previously treated for cancer or diseases or disorders associated with, or characterized by, undesired angiogenesis, but are non-responsive to standard therapies, as well as those who have not previously been treated. The invention also encompasses methods of treating patients regardless of patient's age, although some diseases or disorders are more common in certain age groups. The invention further encompasses methods of treating patients who have undergone surgery in an attempt to treat the disease or condition at issue, as well as those who have not. Because patients with cancer and diseases and disorders characterized by undesired angiogenesis have heterogenous clinical manifestations and varying clinical outcomes, the treatment given to a patient may vary, depending on his/her prognosis. The skilled clinician will be able to readily determine without undue experimentation specific secondary agents, types of surgery, and types of non-drug based standard therapy that can be effectively used to treat an individual patient with cancer and other diseases or disorders.

Methods encompassed by this invention comprise administering one or more immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, to a patient (e.g., a human) suffering, or likely to suffer, from cancer or a disease or disorder mediated by undesired angiogenesis.

In one embodiment of the invention, an immunomodulatory compound of the invention can be administered orally in single or divided daily doses in an amount of from about 0.10 to about 150 mg/day. In a particular embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of from about 0.1 to about 1 mg per day, or alternatively from about 0.1 to about 5 mg every other day. In a preferred embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered in an amount of from about 5 to 25 mg per day, or alternatively from about 10 to about 50 mg every other day.

In a specific embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of about 1, 2, or 5 mg per day to patients with relapsed multiple myeloma. In a particular embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered initially in an amount of 5 mg/day and the dose can be escalated every week to 10, 20, 25, 30 and 50 mg/day. In a specific embodiment, Revimid™ can be administered in an amount of up to about 30 mg/day to patients with solid tumor. In a particular embodiment, Revimid™ can be administered in an amount of up to about 40 mg/day to patients with glioma.

#### 5.3.1 Combination Therapy with a Second Active Agent

Specific methods of the invention comprise administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with one or more second active agents, and/or in combination with radia-

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tion therapy, blood transfusions, or surgery. Examples of immunomodulatory compounds of the invention are disclosed herein (see, e.g., section 5.1). Examples of second active agents are also disclosed herein (see, e.g., section 5.2).

Administration of the immunomodulatory compounds and the second active agents to a patient can occur simultaneously or sequentially by the same or different routes of administration. The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. A preferred route of administration for an immunomodulatory compound of the invention is orally. Preferred routes of administration for the second active agents or ingredients of the invention are known to those of ordinary skill in the art. See, e.g., *Physicians' Desk Reference*, 1755-1760 (56<sup>th</sup> ed., 2002).

In one embodiment of the invention, the second active agent is administered intravenously or subcutaneously and once or twice daily in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. The specific amount of the second active agent will depend on the specific agent used, the type of disease being treated or managed, the severity and stage of disease, and the amount(s) of immunomodulatory compounds of the invention and any optional additional active agents concurrently administered to the patient. In a particular embodiment, the second active agent is oblimersen (Genasense®), GM-CSF, G-CSF, EPO, taxotere, irinotecan, dacarbazine, transretinoic acid, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, vincristine, doxorubicin, COX-2 inhibitor, IL2, IL8, IL18, IFN, Ara-C, vinorelbine, or a combination thereof.

In a particular embodiment, GM-CSF, G-CSF or EPO is administered subcutaneously during about five days in a four or six week cycle in an amount of from about 1 to about 750 mg/m<sup>2</sup>/day, preferably in an amount of from about 25 to about 500 mg/m<sup>2</sup>/day, more preferably in an amount of from about 50 to about 250 mg/m<sup>2</sup>/day, and most preferably in an amount of from about 50 to about 200 mg/m<sup>2</sup>/day. In a certain embodiment, GM-CSF may be administered in an amount of from about 60 to about 500 mcg/m<sup>2</sup> intravenously over 2 hours, or from about 5 to about 12 mcg/m<sup>2</sup>/day subcutaneously. In a specific embodiment, G-CSF may be administered subcutaneously in an amount of about 1 mcg/kg/day initially and can be adjusted depending on rise of total granulocyte counts. The maintenance dose of G-CSF may be administered in an amount of about 300 (in smaller patients) or 480 mcg subcutaneously. In a certain embodiment, EPO may be administered subcutaneously in an amount of 10,000 Unit 3 times per week.

In another embodiment, Revimid™ in an amount of about 25 mg/d and dacarbazine in an amount of about from 200 to 1,000 mg/m<sup>2</sup>/d are administered to patients with metastatic malignant melanoma. In a specific embodiment, Revimid™ is administered in an amount of from about 5 to about 25 mg/d to patients with metastatic malignant melanoma whose disease has progressed on treatment with dacarbazine, IL-2 or IFN. In a specific embodiment, Revimid™ is administered to patients with relapsed or refractory multiple myeloma in an amount of about 15 mg/d twice a day or about 30 mg/d four times a day in a combination with dexamethasone.

In another embodiment, an immunomodulatory compound is administered with melphalan and dexamethasone to patients with amyloidosis. In a specific embodiment, an immunomodulatory compound of the invention and steroids can be administered to patients with amyloidosis.

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In another embodiment, an immunomodulatory compound is administered with gemcitabine and cisplatin to patients with locally advanced or metastatic transitional cell bladder cancer.

In another embodiment, an immunomodulatory compound is administered in combination with a second active ingredient as follows: temozolomide to pediatric patients with relapsed or progressive brain tumors or recurrent neuroblastoma; celecoxib, etoposide and cyclophosphamide for relapsed or progressive CNS cancer; temodar to patients with recurrent or progressive meningioma, malignant meningioma, hemangiopericytoma, multiple brain metastases, relapsed brain tumors, or newly diagnosed glioblastoma multiformis; irinotecan to patients with recurrent glioblastoma; carboplatin to pediatric patients with brain stem glioma; procarbazine to pediatric patients with progressive malignant gliomas; cyclophosphamide to patients with poor prognosis malignant brain tumors, newly diagnosed or recurrent glioblastoma multiformis; Gliadel® for high grade recurrent malignant gliomas; temozolomide and tamoxifen for anaplastic astrocytoma; or topotecan for gliomas, glioblastoma, anaplastic astrocytoma or anaplastic oligodendroglioma.

In another embodiment, an immunomodulatory compound is administered with methotrexate and cyclophosphamide to patients with metastatic breast cancer.

In another embodiment, an immunomodulatory compound is administered with temozolomide to patients with neuroendocrine tumors.

In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with recurrent or metastatic head or neck cancer. In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with pancreatic cancer.

In another embodiment, an immunomodulatory compound is administered to patients with colon cancer in combination with Arisa®, taxol and/or taxotere.

In another embodiment, an immunomodulatory compound is administered with capecitabine to patients with refractory colorectal cancer or patients who fail first line therapy or have poor performance in colon or rectal adenocarcinoma.

In another embodiment, an immunomodulatory compound is administered in combination with fluorouracil, leucovorin, and irinotecan to patients with Dukes C & D colorectal cancer or to patients who have been previously treated for metastatic colorectal cancer.

In another embodiment, an immunomodulatory compound is administered to patients with refractory colorectal cancer in combination with capecitabine, xeloda, and/or CPT-11.

In another embodiment, an immunomodulatory compound of the invention is administered with capecitabine and irinotecan to patients with refractory colorectal cancer or to patients with unresectable or metastatic colorectal carcinoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with interferon alpha or capecitabine to patients with unresectable or metastatic hepatocellular carcinoma; or with cisplatin and thiopeta to patients with primary or metastatic liver cancer.

In another embodiment, an immunomodulatory compound is administered in combination with pegylated interferon alpha to patients with Kaposi's sarcoma.

In another embodiment, an immunomodulatory compound is administered in combination with fludarabine, carboplatin, and/or topotecan to patients with refractory or relapsed or high-risk acuted myelogenous leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with liposomal daunorubicin,

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topotecan and/or cytarabine to patients with unfavorable karyotype acute myeloblastic leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with gemcitabine and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered in combination with carboplatin and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered with doxetaxol to patients with non-small cell lung cancer who have been previously treated with carbo/VP 16 and radiotherapy.

In another embodiment, an immunomodulatory compound is administered in combination with carboplatin and/or taxotere, or in combination with carboplatin, paclitaxel and/or thoracic radiotherapy to patients with non-small cell lung cancer. In a specific embodiment, an immunomodulatory compound is administered in combination with taxotere to patients with stage IIIB or IV non-small cell lung cancer.

In another embodiment, an immunomodulatory compound of the invention is administered in combination with oblimersen (Genasense®) to patients with small cell lung cancer.

In another embodiment, an immunomodulatory compound is administered alone or in combination with a second active ingredient such as vinblastine or fludarabine to patients with various types of lymphoma, including, but not limited to, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma or relapsed or refractory low grade follicular lymphoma.

In another embodiment, an immunomodulatory compound is administered in combination with taxotere, IL-2, IFN, GM-CSF, and/or dacarbazine to patients with various types or stages of melanoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with vinorelbine to patients with malignant mesothelioma, or stage IIIB non-small cell lung cancer with pleural implants or malignant pleural effusion mesothelioma syndrome.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of multiple myeloma in combination with dexamethasone, zoledronic acid, palmitronate, GM-CSF, biacin, vinblastine, melphalan, busulphan, cyclophosphamide, IFN, palmidronate, prednisone, bisphosphonate, celecoxib, arsenic trioxide, PEG INTRON-A, vincristine, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with relapsed or refractory multiple myeloma in combination with doxorubicin (Doxil®), vincristine and/or dexamethasone (Decadron®).

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of ovarian cancer such as peritoneal carcinoma, papillary serous carcinoma, refractory ovarian cancer or recurrent ovarian cancer, in combination with taxol, carboplatin, doxorubicin, gemcitabine, cisplatin, xeloda, paclitaxel, dexamethasone, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of prostate cancer, in combination with xeloda, 5 FU/LV, gemcitabine, irinotecan plus gemcitabine, cyclophosphamide, vincristine, dexamethasone, GM-CSF, celecoxib, taxotere, ganciclovir, paclitaxel, adriamycin, docetaxel, estramustine, Emcyt, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of

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renal cell cancer, in combination with capecitabine, IFN, tamoxifen, IL-2, GM-CSF, Celebrex®, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of gynecologic, uterus or soft tissue sarcoma cancer in combination with IFN, a COX-2 inhibitor such as Celebrex®, and/or sulindac.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of solid tumors in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apecitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with scleroderma or cutaneous vasculitis in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apecitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

This invention also encompasses a method of increasing the dosage of an anti-cancer drug or agent that can be safely and effectively administered to a patient, which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable derivative, salt, solvate, clathrate, hydrate, or pro-drug thereof. Patients that can benefit by this method are those likely to suffer from an adverse effect associated with anti-cancer drugs for treating a specific cancer of the skin, subcutaneous tissue, lymph nodes, brain, lung, liver, bone, intestine, colon, heart, pancreas, adrenal, kidney, prostate, breast, colorectal, or combinations thereof. The administration of an immunomodulatory compound of the invention alleviates or reduces adverse effects which are of such severity that it would otherwise limit the amount of anti-cancer drug.

In one embodiment, an immunomodulatory compound of the invention can be administered orally and daily in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 50 mg, more preferably from about 2 to about 25 mg prior to, during, or after the occurrence of the adverse effect associated with the administration of an anti-cancer drug to a patient. In a particular embodiment, an immunomodulatory compound of the invention is administered in combination with specific agents such as heparin, aspirin, coumadin, or G-CSF to avoid adverse effects that are associated with anti-cancer drugs such as but not limited to neutropenia or thrombocytopenia.

In one embodiment, an immunomodulatory compound of the invention can be administered to patients with diseases and disorders associated with, or characterized by, undesired angiogenesis in combination with additional active ingredients including but not limited to anti-cancer drugs, anti-inflammatories, antihistamines, antibiotics, and steroids.

In another embodiment, this invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with (e.g. before, during, or after) conventional therapy including, but not limited to, surgery, immunotherapy, biological therapy, radiation therapy, or other non-drug based therapy presently used to treat, prevent or manage cancer. The combined use of the immunomodulatory compounds of the invention and conventional therapy may provide a unique treatment regimen that is unexpectedly effective in certain patients. Without being limited by theory, it is believed that immunomodulatory compounds of the invention may provide additive or synergistic effects when given concurrently with conventional therapy.



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As discussed elsewhere herein, the invention encompasses a method of reducing, treating and/or preventing adverse or undesired effects associated with conventional therapy including, but not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy. One or more immunomodulatory compounds of the invention and other active ingredient can be administered to a patient prior to, during, or after the occurrence of the adverse effect associated with conventional therapy.

In one embodiment, an immunomodulatory compound of the invention can be administered in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 25 mg, more preferably from about 2 to about 10 mg orally and daily alone, or in combination with a second active agent disclosed herein (see, e.g., section 5.2), prior to, during, or after the use of conventional therapy.

In a specific embodiment of this method, an immunomodulatory compound of the invention and doxorubicin are administered to patients with non-small cell lung cancer who were previously treated with carboplatin and radiotherapy.

#### 5.3.2 Use with Transplantation Therapy

Compounds of the invention can be used to reduce the risk of Graft Versus Host Disease (GVHD). Therefore, the invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering the immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with transplantation therapy.

As those of ordinary skill in the art are aware, the treatment of cancer is often based on the stages and mechanism of the disease. For example, as inevitable leukemic transformation develops in certain stages of cancer, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. The combined use of the immunomodulatory compound of the invention and transplantation therapy provides a unique and unexpected synergism. In particular, an immunomodulatory compound of the invention exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy in patients with cancer.

An immunomodulatory compound of the invention can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of GVHD. This invention encompasses a method of treating, preventing and/or managing cancer which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after the transplantation of umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow. Examples of stem cells suitable for use in the methods of the invention are disclosed in U.S. provisional patent application No. 60/372,348, filed Apr. 12, 2002 by R. Hariri et al., the entirety of which is incorporated herein by reference.

In one embodiment of this method, an immunomodulatory compound of the invention is administered to patients with multiple myeloma before, during, or after the transplantation of autologous peripheral blood progenitor cell.

In another embodiment, an immunomodulatory compound is administered to patients with relapsing multiple myeloma after the stem cell transplantation.

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In another embodiment, an immunomodulatory compound and prednisone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous stem cell.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as salvage therapy for low risk post transplantation to patients with multiple myeloma.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous bone marrow.

In another embodiment, an immunomodulatory compound is administered following the administration of high dose of melphalan and the transplantation of autologous stem cell to patients with chemotherapy responsive multiple myeloma.

In another embodiment, an immunomodulatory compound and PEG INTRO-A are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous CD34-selected peripheral stem cell.

In another embodiment, an immunomodulatory compound is administered with post transplant consolidation chemotherapy to patients with newly diagnosed multiple myeloma to evaluate anti-angiogenesis.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy after DCEP consolidation, following the treatment with high dose of melphalan and the transplantation of peripheral blood stem cell to 65 years of age or older patients with multiple myeloma.

#### 5.3.3 Cycling Therapy

In certain embodiments, the prophylactic or therapeutic agents of the invention are cyclically administered to a patient. Cycling therapy involves the administration of an active agent for a period of time, followed by a rest for a period of time, and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

Consequently, in one specific embodiment of the invention, an immunomodulatory compound of the invention is administered daily in a single or divided doses in a four to six week cycle with a rest period of about a week or two weeks. The invention further allows the frequency, number, and length of dosing cycles to be increased. Thus, another specific embodiment of the invention encompasses the administration of an immunomodulatory compound of the invention for more cycles than are typical when it is administered alone. In yet another specific embodiment of the invention, an immunomodulatory compound of the invention is administered for a greater number of cycles that would typically cause dose-limiting toxicity in a patient to whom a second active ingredient is not also being administered.

In one embodiment, an immunomodulatory compound of the invention is administered daily and continuously for three or four weeks at a dose of from about 0.1 to about 150 mg/d followed by a break of one or two weeks. Actimid™ is preferably administered daily and continuously at an initial dose of 0.1 to 5 mg/d with dose escalation (every week) by 1 to 10 mg/d to a maximum dose of 50 mg/d for as long as therapy is tolerated. In a particular embodiment, Revimid™ is administered in an amount of about 5, 10, or 25 mg/day, preferably in an amount of about 10 mg/day for three to four weeks, followed by one week or two weeks of rest in a four or six week cycle.

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In one embodiment of the invention, an immunomodulatory compound of the invention and a second active ingredient are administered orally, with administration of an immunomodulatory compound of the invention occurring 30 to 60 minutes prior to a second active ingredient, during a cycle of four to six weeks. In another embodiment of the invention, the combination of an immunomodulatory compound of the invention and a second active ingredient is administered by intravenous infusion over about 90 minutes every cycle. In a specific embodiment, one cycle comprises the administration of from about 10 to about 25 mg/day of Revimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient daily for three to four weeks and then one or two weeks of rest. In another specific embodiment, each cycle comprises the administration of from about 5 to about 10 mg/day of Actimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient for 3 to 4 weeks followed by one or two weeks of rest. Typically, the number of cycles during which the combinatorial treatment is administered to a patient will be from about one to about 24 cycles, more typically from about two to about 16 cycles, and even more typically from about four to about three cycles.

#### 5.4 Pharmaceutical Compositions and Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Pharmaceutical compositions and dosage forms of the invention can further comprise one or more excipients.

Pharmaceutical compositions and dosage forms of the invention can also comprise one or more additional active ingredients. Consequently, pharmaceutical compositions and dosage forms of the invention comprise the active ingredients disclosed herein (e.g., an immunomodulatory compound and a second active agent). Examples of optional second, or additional, active ingredients are disclosed herein (see, e.g., section 5.2).

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), topical (e.g., eye drops or other ophthalmic preparations), transdermal or transcutaneous administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; eye drops or other ophthalmic preparations suitable for topical administration; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease. These and other ways in which

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specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients may be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose other mono- or disaccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the *U.S. Pharmacopeia* (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples

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of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as “stabilizers,” include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of from about 0.10 to about 150 mg. Typical dosage forms comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of about 0.1, 1, 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 50, 100, 150 or 200 mg. In a particular embodiment, a preferred dosage form comprises 4-(amino-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) in an amount of about 1, 2, 5, 10, 25 or 50 mg. In a specific embodiment, a preferred dosage form comprises 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) in an amount of about 5, 10, 25 or 50 mg. Typical dosage forms comprise the second active ingredient in an amount of 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. Of course, the specific amount of the anti-cancer drug will depend on the specific agent used, the type of cancer being treated or managed, and the amount(s) of an immunomodulatory compound of the invention and any optional additional active agents concurrently administered to the patient.

#### 5.4.1 Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and inti-

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ately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene



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glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

A preferred solid oral dosage form of the invention comprises an immunomodulatory compound of the invention, anhydrous lactose, microcrystalline cellulose, polyvinylpyrrolidone, stearic acid, colloidal anhydrous silica, and gelatin.

#### 5.4.2 Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

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#### 5.4.3 Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention. For example, cyclodextrin and its derivatives can be used to increase the solubility of an immunomodulatory compound of the invention and its derivatives. See, e.g., U.S. Pat. No. 5,134,127, which is incorporated herein by reference.

#### 5.4.4 Topical and Mucosal Dosage Forms

Topical and mucosal dosage forms of the invention include, but are not limited to, sprays, aerosols, solutions, emulsions, suspensions, eye drops or other ophthalmic preparations, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide topical and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form solutions, emulsions or gels, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990).

The pH of a pharmaceutical composition or dosage form may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-

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enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

#### 5.4.5 Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a dosage form of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, pro-drug, or clathrate thereof. Kits encompassed by this invention can further comprise additional active ingredients such as oblimersen (Genasense®), melphalan, G-CSF, GM-CSF, EPO, topotecan, dacarbazine, irinotecan, taxotere, IFN, COX-2 inhibitor, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13 cis-retinoic acid, or a pharmacologically active mutant or derivative thereof, or a combination thereof. Examples of the additional active ingredients include, but are not limited to, those disclosed herein (see, e.g., section 5.2).

Kits of the invention can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

## 6. EXAMPLES

Certain embodiments of the invention are illustrated by the following non-limiting examples.

### 6.1 Modulation of Cytokine Production

A series of non-clinical pharmacology and toxicology studies have been performed to support the clinical evaluation of an immunomodulatory compound of the invention in human subjects. These studies were performed in accordance with internationally recognized guidelines for study design and in compliance with the requirements of Good Laboratory Practice (GLP), unless otherwise noted.

Inhibition of TNF- $\alpha$  production following LPS-stimulation of human PBMC and human whole blood by 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™), 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione and thalidomide (Revimid™) was investigated in vitro (Muller et al., *Bioorg. Med. Chem. Lett.* 9:1625-1630, 1999). The IC<sub>50</sub>'s of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and

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human whole blood were ~24 nM (6.55 ng/mL) and ~25 nM (6.83 ng/mL), respectively. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but at least 200 times more potent than, thalidomide. In vitro studies have also demonstrated that concentrations of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione of 2.73 to 27.3 ng/mL (0.01 to 0.1  $\mu$ M) achieved 50% inhibition of the proliferation of MM.1S and Hs Sultan cells.

The IC<sub>50</sub>'s of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and human whole blood were ~100 nM (25.9 ng/mL) and ~480 nM (103.6 ng/mL), respectively. Thalidomide, in contrast, had an IC<sub>50</sub> of ~194  $\mu$ M (50.2  $\mu$ g/mL) for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but 50 to 2000 times more potent than, thalidomide. It has been shown that the compound is approximately 50-100 times more potent than thalidomide in stimulating the proliferation of T-cells following primary induction by T-cell receptor (TCR) activation. 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is also approximately 50 to 100 times more potent than thalidomide in augmenting the production of IL-2 and IFN- $\gamma$  following TCR activation of PBMC (IL-2) or T-cells (IFN- $\gamma$ ). In addition, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione exhibited dose-dependent inhibition of LPS-stimulated production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 by PBMC while it increased production of the anti-inflammatory cytokine IL-10.

### 6.2 Inhibition of MM Cell Proliferation

The ability of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide for comparison to effect the proliferation of MM cell lines has been investigated in an in vitro study. Uptake [<sup>3</sup>H]-thymidine by different MM cell lines (MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of cell proliferation. Cells were incubated in the presence of compounds for 48 hours; [<sup>3</sup>H]-thymidine was included for the last 8 hours of the incubation period. Addition of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione to MM.1S and Hs Sultan cells resulted in 50% inhibition of cell proliferation at concentrations of 0.4  $\mu$ M and 1  $\mu$ M, respectively. In contrast, addition of thalidomide at concentrations up to 100  $\mu$ M resulted in only 15% and 20% inhibition of cell proliferation in MM.1S and Hs Sultan cells, respectively. These data are summarized in FIG. 1.

### 6.3 Toxicology Studies

The effects of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) on cardiovascular and respiratory function are investigated in anesthetized dogs. Two groups of Beagle dogs (2/sex/group) are used. One group receives three doses of vehicle only and the other receives three ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (2, 10, and 20 mg/kg). In all cases, doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione or vehicle are successively administered via infusion through the jugular vein separated by intervals of at least 30 minutes.

The cardiovascular and respiratory changes induced by 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione are minimal at all doses when compared to the vehicle control group. The only statistically significant difference between the vehicle and treatment groups is a small increase in arterial blood pressure (from 94 mmHg to 101

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mmHg) following administration of the low dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. This effect lasts approximately 15 minutes and is not seen at higher doses. Deviations in femoral blood flow, respiratory parameters, and Qtc interval are common to both the control and treated groups and are not considered treatment-related.

#### 6.4 Cycling Therapy in Patients

In a specific embodiment, an immunomodulatory compound of the invention are cyclically administered to patients with cancer. Cycling therapy involves the administration of a first agent for a period of time, followed by a rest for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

In a specific embodiment, prophylactic or therapeutic agents are administered in a cycle of about 4 to 6 weeks, about once or twice every day. One cycle can comprise the administration of a therapeutic or prophylactic agent for three to four weeks and at least a week or two weeks of rest. The number of cycles administered is from about one to about 24 cycles, more typically from about two to about 16 cycles, and more typically from about four to about eight cycles.

For example, in a cycle of four weeks, on day 1, the administration of 25 mg/d of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is started. On day 22, the administration of the compound is stopped for a week of rest. On day 29, the administration of 25 mg/d 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is begun.

#### 6.5 Clinical Studies in Patients

##### 6.5.1 Treatment of Relapsed Multiple Myeloma

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) was administered to patients with relapsed/refractory multiple myeloma. The study was conducted in compliance with Good Clinical Practices. Patients were at least 18 years old, had been diagnosed with multiple myeloma (with paraprotein in serum and/or urine), and were considered refractory to treatment after at least two cycles of treatment, or have relapsed after two cycles of treatment.

Patients who have progressive disease, according to the Southwest Oncology Group (SWOG) criteria, on their prior regimen are considered treatment refractory. Relapse following remission is defined as >25% increase in M component from baseline levels; reappearance of the M paraprotein that had previously disappeared; or a definite increase in the size and number of lytic bone lesions recognized on radiographs. Patients may have had prior therapy with thalidomide, provided they were able to tolerate the treatment. A Zubrod performance status of 0 to 2 is required for all patients.

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione is administered to patients at doses of 1, 2, 5, or 10 mg/day for up to four weeks; at each dose level, three patients are initially enrolled. Dosing occurs at approximately the same time each morning; all doses are administered in the fasted state (no eating for at least two hours prior to dosing and two hours after dosing). 4-(amino)-2-(2,6-dioxo(3-piperidyl))-

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isoindoline-1,3-dione doses are administered in an ascending fashion such that patients in the first cohort receive the lowest dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (1 mg/day) and escalation to the next higher dose level occurs only following the establishment of safety and tolerability at the current dose. If one out of three patients at any dose level experience dose limiting toxicity (DLT), three additional patients are enrolled at that dose. If none of the three additional patients experience DLT, escalation to the next dose level occurs; dose escalations continue in a similar fashion until the MTD is established or the maximum daily dose (10 mg/day) is attained. However, if one of the three additional patients enrolled experiences DLT, the MTD has been reached. If two or more of the three additional patients enrolled experience DLT, the MTD is judged to have been exceeded and three additional patients are enrolled at the preceding dose level to confirm the MTD. Once the MTD has been identified, four additional patients are enrolled at that dose level so that a total of 10 patients is treated at the MTD.

Blood sampling for analysis of pharmacokinetic parameters is performed on Days 1 and 28 according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. An additional blood sample is collected at each weekly visit for the determination of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione levels. Total urine collections are also made with urine pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Safety assessments are made by monitoring adverse events, vital signs, ECGs, clinical laboratory evaluations (blood chemistry, hematology, lymphocyte phenotyping, and urinalysis), and physical examination at specific times during the study.

Results of interim pharmacokinetic analyses obtained following single- and multiple-dose administration of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione to multiple myeloma patients are presented below in Tables 1 and 2. These data show that 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione was steadily absorbed at all dose levels in relapsed multiple myeloma patients. Maximum plasma concentrations occurred at a median  $T_{max}$  of between 2.5 and 2.8 hours post-dose at Day 1 and between 3 and 4 hours post-dose at Week 4. At all doses, plasma concentrations declined in a monophasic manner after reaching  $C_{max}$ . The start of the elimination phase occurred between 3 and 10 hours post-dose at Day 1 and Week 4, respectively.

These data also showed that after 4 weeks of dosing, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione accumulated to a small extent (mean accumulation ratios ~1.02 to 1.52 and ~0.94 to 1.62 for  $C_{max}$  and  $AUC_{(0-\tau)}$ , respectively). There was almost a dose proportional increase in  $AUC_{(0-\tau)}$  and  $C_{max}$  values with increasing dose. A five-fold higher dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione produced a 3.2- and 2.2-fold increase in  $C_{max}$  at Day 1 and Week 4, respectively. Similarly, a 5-fold increase in dose resulted in a 3.6- and 2.3-fold increase in  $AUC_{(0-\tau)}$ , at Day 1 and Week 4, respectively.

TABLE 1

Pharmacokinetic parameters of Actimid™ in relapsed multiple myeloma patients				
Parameter		1 mg (N = 6)	2 mg (N = 2)	5 mg (N = 3)
		Day 1		
$C_{max}$	ng/mL	15.03 (4.04)	24.4* (12.1)	48.56 (14.03)
$t_{max}$	h	3.3 (2.6)	2.7* (0.3)	2.3 (0.3)

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TABLE 1-continued

Pharmacokinetic parameters of Actimid™ in relapsed multiple myeloma patients				
Parameter		1 mg (N = 6)	2 mg (N = 2)	5 mg (N = 3)
Day 1				
AUC <sub>(0-∞)</sub>	ng · h/mL	152.90 (36.62)	279.18 (51.10)	593.10 (335.23)
AUC <sub>(0-τ)</sub>		134.21 (27.14)	249.57 (29.26)	520.94 (267.32)
t <sub>1/2</sub>	h	7.3 (3.4)	6.3 (1.4)	6.5 (2.2)
CL/F	mL/min	114.75 (29.20)	121.43 (22.22)	182.31 (117.06)
Vz/f	L	69.55 (44.97)	65.31 (2.80)	87.24 (22.61)

τ = 24 hours

N/A = not available

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TABLE 2

Pharmacokinetic parameters of Actimid™ following multiple oral doses(1, 2, and 5 mg/day) in relapsed multiple myeloma patients				
Parameter		1 mg (N = 5)	2 mg (N = 2)	5 mg (N = 3)
Week 4				
C <sub>max</sub>	ng/mL	23.20 (7.48)	30.05* (15.64)	58.07 (38.08)
t <sub>max</sub>	h	3.6 (1.5)	2.8* (0.3)	5.0 (2.6)
AUC <sub>(0-∞)</sub>	ng · h/mL	N/A	N/A	N/A
AUC <sub>(0-τ)</sub>		239.31 (122.59)	269.36 (186.34)	597.24 (354.23)
t <sub>1/2</sub>	h	6.2* (0.6)	7.7 (2.8)	7.8 (4.0)
CL/F	mL/min	87.85 (48.48)	162.68 (112.54)	207.50 (175.41)
Vz/f	L	41.35* (8.84)	95.04 (35.39)	103.95 (27.25)

τ = 24 hours

N/A = not available

\*N = 3 patients

### 6.5.2 Treatment of Relapsed Multiple Myeloma

Two Phase 1 clinical studies of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) have been conducted to identify the maximum tolerated dose (MTD) in patients with refractory or relapsed multiple myeloma. These studies have also characterized the safety profile of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione when ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione were given orally for up to 4 weeks. Patients started 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment at 5 mg/day with subsequent escalation to 10, 25, and 50 mg/day. Patients were enrolled for 28 days at their assigned dose, with the option of extended treatment for those who did not exhibit disease progression or experience dose limiting toxicity (DLT). Patients were evaluated for adverse events at each visit and the severity of these events was graded according to the National Cancer Institute (NCI) Common Toxicity Criteria. Patients were discontinued if they experienced DLT (Grade 3 or greater non-hematological, or Grade 4 hematological toxicity).

In this study, 27 patients were enrolled. All patients had relapsed multiple myeloma and 18 (72%) were refractory to salvage therapy. Among these patients, 15 had undergone prior autologous stem cell transplantation and 16 patients had received prior thalidomide treatment. The median number of prior regimens was 3 (range 2 to 6).

Blood and urine samples were collected for analysis of pharmacokinetic parameters on Days 1 and 28. Blood samples were collected according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. In addition, a blood sample was collected at each weekly clinic visit for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione deter-

mination. Total urine was collected and pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Response to treatment was assessed by M-protein quantification (by immunoelectrophoresis) from serum and a 24-hour urine collection, with creatinine clearance and 24-hour protein calculations undertaken at screening, baseline, Weeks 2 and 4, and monthly thereafter (or upon early termination). Bone marrow aspirations and/or tissue biopsy are also performed at Months 3, 6 and 12 if a patient's paraprotein serum concentration or 24-hour urine protein excretion declined to the next lower level, based on best response criteria. Preliminary results for the 28-day treatment period are summarized below.

Preliminary pharmacokinetic analyses based on these two studies indicated that AUC and C<sub>max</sub> values increase proportionally with dose following single and multiple doses in multiple myeloma patients (as was seen in healthy volunteers). Further, there was no evidence of accumulation with multiple dosing as single dose AUC<sub>(0-∞)</sub> was comparable to multiple dose AUC<sub>(0-τ)</sub> following the same dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione.

Similar to healthy volunteer studies, double peaks were observed. Exposure in multiple myeloma patients appeared to be slightly higher based on C<sub>max</sub> and AUC values as compared to healthy male volunteers while clearance in multiple myeloma patients was lower than it was in healthy volunteers, consistent with their poorer renal function (both as a consequence of their age and their disease). Finally, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione half-life in patients was shorter than in healthy volunteers (mean 8 hours, ranging up to 17 hours).

In this study, the first cohort of 3 patients was treated for 28 days at 5 mg/day without any dose limiting toxicity (DLT). The second cohort of 3 patients subsequently commenced



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therapy at 10 mg/day. Patients in the second 10 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione cohort tolerated treatment well.

#### 6.5.3 Treatment of Solid Tumors

Study with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) was conducted in patients with varying types of solid tumors, including malignant melanoma (13), carcinoma of the pancreas (2), carcinoid-unknown primary (1), renal carcinoma (1), breast carcinoma (1) and NSCLC (2). Patients received 5 mg/day 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for seven days and are subsequently escalated every seven days to 10 mg/day, 25 mg/day, and 50 mg/day for a total of 4 weeks of treatment. Patients who, experienced clinical benefit were permitted to continue on treatment as Named Patients.

The study initially enrolled 20 patients and was subsequently amended to enroll 16 additional patients (adrenal carcinoma, NSCLC, malignant mesothelioma, breast cancer, malignant melanoma (8), renal cell cancer (4)) at a higher dose. The 16 additional patients were given weekly escalating doses of 25 mg/day, 50 mg/day, 75 mg/day, 100 mg/day, 125 mg/day, and 150 mg/day over a 6-week period with continuing treatment for an additional six weeks.

The study of Phase 1 study was designed to determine a maximum tolerated dose (MTD) of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in patients with refractory solid tumors and/or lymphoma, as well as to characterize the pharmacokinetic and side effect profiles of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in this patient population. The study design dictates that at least 3 patients must be enrolled at a dose level and have completed 28 days of treatment prior to enrollment of patients at the next higher dose level. Patients in the first cohort began dosing at 5 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Patients will be escalated to 10, 20, 25, and 30 mg/day provided there is no toxicity.

In this study, the MTD is defined as the highest dose level in which fewer than two of six patients treated did not experience Grade 3 or greater non-hematological toxicity or Grade 4 or greater hematological toxicity. If, at any given dose level in either study, one out of three patients experiences toxicity, three additional patients must be treated at that particular dose. If, however, two out of six patients experience DLT, the MTD is judged to have been exceeded. No further dose escalations are to occur and additional patients are to be enrolled at the previous dose level. The dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered is escalated until the MTD is achieved or the maximum daily dose of is reached.

No DLTs were reported in the initial group of 20 patients enrolled in the study. Thirteen of the original 20 trial patients, along with 2 non-trial patients, continued on treatment as named patients at doses up to 150 mg/day.

#### 6.5.4 Treatment of Gliomas

This study was performed to find toxicity in patients with recurrent, high-grade gliomas. The study is designed such that patients are given increasingly higher doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione until a maximum tolerated dose (MTD) is established. The study also seeks to obtain preliminary toxicity information and pharmacokinetic data on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, as well as to develop exploratory data concerning surrogate end points of angiogenic activity in vivo using functional neuro-imaging studies, and in vitro assays of serum angiogenic peptides.

Patients enrolled in the first cohort receive 2.5 mg/m<sup>2</sup>/day for a 4-week cycle. During each 4-week cycle of therapy,

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3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered once daily for 3 weeks followed by a week of rest. Patients who complete a treatment cycle may receive another cycle of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment if two criteria are met. First, the patient must have stable disease or have experienced a partial response or complete response, or the patient is benefiting from the therapy with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione as evidenced by a decrease in tumor-related symptoms such as neurological deficits. Second, the patient must have recovered from toxicity related to 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione which occurred in the prior cycle by Day 42 or sooner (28-day cycle plus limit of 2 weeks to recover) as evidenced by a return to Grade ≤1 toxicity level. Patients who experience DLT in the previous cycle should have their dose modified. DLT is defined as a non-hematological event Grade ≥3 toxicity or hematological event of Grade 4 toxicity thought to be related to the study medication. Patients who experience DLT in the first cycle and have no response to therapy are removed from the study.

3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione doses are subsequently escalated to 5, 8, 11, 15, and 20 mg/m<sup>2</sup>/day to a maximum total daily dose of 40 mg. Patients continue to receive 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione on a 4-week cycle per dose level until one of the off-study criteria are met.

Three patients are enrolled in each cohort. If at least one DLT occurs, three additional patients are added to the cohort at that particular dose level. If two DLTs occur, the MTD, defined as the dose at which fewer than one-third of patients at each dose level experiences DLT has been exceeded and four more patients are treated at the previous dose.

Patients who experience DLT during the first 4-week cycle are removed from the study, except if they have a response to therapy. For patients who have completed their first 4-week cycle of without DLT, but who subsequently experience Grade 3 or 4 hematological and/or nonhematological toxicity, treatment is suspended for a minimum of a week. If the toxicity resolves to <Grade 2 within three weeks, the patient is treated at two dose levels lower than the dose that caused the toxicity (or a 50% reduction if the patient was treated at the first or second dose level). Patients in whom Grade 3 or 4 toxicity does not resolve to <Grade 1 within three weeks, or those who have another Grade 3 toxicity at the reduced dose are removed from the study.

Pharmacokinetic sampling is performed prior the first dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Day 1) and 0.5, 1, 2, 4, 6, 8, 24, and 48 hours thereafter. Sampling is also conducted pre-dose on Days 7 and 21 and 0.5, 1, 2, 4, 6, 8, and 24 post-dose on Day 21 to evaluate steady-state 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione levels.

#### 6.5.5 Treatment of Metastatic Melanoma

Patients with metastatic melanoma were started on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) at 5 mg/day for seven days. The dose was then increased every seven days to 10 mg/day, 25 mg/day, and 50 mg/day, respectively, for a total of four weeks on therapy. Five of the 13 melanoma patients who were treated under this regimen either showed disease stabilization or a partial response in the first four weeks of treatment. Tumor response was seen in cutaneous and subcutaneous lesions (five patients), lymph nodes (two patients), and liver (one patient). The duration of response was approximately six months. The

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result suggests that the compound appears is a promising new anti-cancer agent and has both antiangiogenic and immunomodulatory properties.

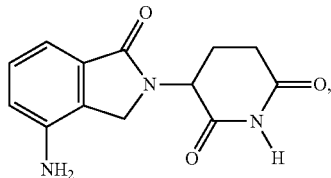
#### 6.5.6 Treatment of Relapsed or Refractory Multiple Myeloma

Patients with relapsed and refractory Dune-Salmon stage III multiple myeloma, who have either failed at least three previous regimens or presented with poor performance status, neutropenia or thrombocytopenia, are treated with up to four cycles of combination of melphalan (50 mg intravenously), an immunomodulatory compound of the invention (about 1 to 150 mg orally daily), and dexamethasone (40 mg/day orally on days 1 to 4) every four to six weeks. Maintenance treatment consisting of daily an immunomodulatory compound of the invention and monthly dexamethasone are continued until the disease progression. The therapy using an immunomodulatory compound of the invention in combination with melphalan and dexamethasone is highly active and generally tolerated in heavily pretreated multiple myeloma patients whose prognosis is otherwise poor.

The embodiments of the invention described above are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials, and procedures. All such equivalents are considered to be within the scope of the invention and are encompassed by the appended claims.

What is claimed is:

1. A method of treating multiple myeloma, which comprises cyclically administering to a patient having multiple myeloma: (a) about 1 to about 50 mg per day of a compound having the formula:



or a pharmaceutically acceptable salt, solvate or stereoisomer thereof for 21 consecutive days followed by seven consecutive days of rest from administration of said compound in a 28 day cycle, and (b) a therapeutically effective amount of dexamethasone.

2. The method of claim 1, wherein the multiple myeloma is newly diagnosed multiple myeloma, smoldering multiple myeloma, refractory multiple myeloma, relapsed multiple myeloma, or relapsed and refractory multiple myeloma.

3. The method of claim 1, wherein the compound is a pharmaceutically acceptable salt.

4. The method of claim 1, wherein the compound is a pharmaceutically acceptable stereoisomer.

5. The method of claim 4, wherein the stereoisomer is an enantiomerically pure R isomer.

6. The method of claim 4, wherein the stereoisomer is an enantiomerically pure S isomer.

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7. The method of claim 1, which further comprises administering a therapeutically effective amount of an additional active agent.

8. The method of claim 7, wherein the additional active agent is melphalan, doxorubicin, vincristine, prednisone, cyclophosphamide, biacin, a proteasome inhibitor, or a combination thereof.

9. The method of claim 1, which further comprises autologous stem cell transplantation.

10. The method of claim 1, wherein the multiple myeloma is smoldering multiple myeloma.

11. The method of claim 1, wherein the multiple myeloma is refractory multiple myeloma.

12. The method of claim 1, wherein the multiple myeloma is relapsed multiple myeloma.

13. The method of claim 1, wherein the compound is administered in the form of a capsule or tablet.

14. The method of claim 1, wherein the compound is administered in a capsule of 5 mg, 10 mg, 15 mg or 25 mg.

15. The method of claim 1, wherein the compound is administered in an amount of about 25 mg per day.

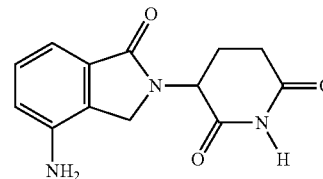
16. The method of claim 1, wherein the compound is administered in an amount of 15 mg per day.

17. The method of claim 1, wherein the compound is administered in an amount of 10 mg per day.

18. The method of claim 1, wherein the compound is administered in an amount of 5 mg per day.

19. The method of claim 1, wherein the dexamethasone is administered in an amount of 40 mg per day on days 1-4 every 28 days.

20. The method of claim 1, wherein the compound is



and is not a pharmaceutically acceptable salt, solvate or stereoisomer hereof.

21. The method of claim 14, wherein the capsule comprises the compound, lactose anhydrous, microcrystalline cellulose, croscarmellose sodium and magnesium stearate.

22. The method of claim 1, wherein the dexamethasone is orally administered in an amount of 40 mg once daily on days 1, 8, 15 and 22 of each 28 day cycle.

23. The method of claim 1, wherein the multiple myeloma is newly diagnosed multiple myeloma.

24. The method of claim 1, wherein the compound is administered in a capsule in an amount of 1 mg to 50 mg.

25. The method of claim 24, wherein the capsule comprises the compound, lactose anhydrous, microcrystalline cellulose, croscarmellose sodium and magnesium stearate.

\* \* \* \* \*



# EXHIBIT H



US009056120B2

(12) **United States Patent**  
**Zeldis**

(10) **Patent No.:** **US 9,056,120 B2**  
(45) **Date of Patent:** **\*Jun. 16, 2015**

(54) **METHODS OF TREATING  
MYELODYSPLASTIC SYNDROMES WITH A  
COMBINATION THERAPY USING  
LENALIDOMIDE AND AZACITIDINE**

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**ABSTRACT**

Methods of treating, preventing and/or managing myelodysplastic syndromes are disclosed. Specific methods encompass the administrations of 3-(4-amino-1-oxo-1,3-dihydroisoindol-2-yl)-piperidin-2,6-dione in combination with 5-azacytidine.

**53 Claims, No Drawings**

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# **METHODS OF TREATING MYELODYSPLASTIC SYNDROMES WITH A COMBINATION THERAPY USING LENALIDOMIDE AND AZACITIDINE**

## **1. CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a continuation application of U.S. patent application Ser. No. 12/777,765, filed May 11, 2010, presently pending, which is continuation-in-part of U.S. patent application Ser. No. 11/985,032, filed Nov. 12, 2007, now issued U.S. Pat. No. 7,863,297, which is continuation of U.S. patent application Ser. No. 11/654,550 filed Jan. 16, 2007, now issued U.S. Pat. No. 7,393,863, which is divisional of U.S. patent application Ser. No. 10/411,649 filed Apr. 11, 2003, now issued U.S. Pat. No. 7,189,740, which claims the benefit of U.S. Provisional Patent Application No. 60/418,468 filed on Oct. 15, 2002, the contents of each of which are incorporated by reference herein in their entireties.

## **2. FIELD**

Provided herein are methods for the treatment of myelodysplastic syndromes ("MDS"), with an immunomodulatory compound, including, but not limited to, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, which is also known as lenalidomide or Revlimid®, in combination with a cytidine analog, including, but not limited to, 5-azacytidine. Also included are methods for improving the overall survival of certain classes of patients having MDS. The invention also encompasses pharmaceutical compositions, dosing regimens, and the use of an immunomodulatory compound and 5-azacytidine in conjunction with transplantation therapy and/or other standard therapies for myelodysplastic syndromes.

## **3. BACKGROUND**

### **3.1. Pathobiology of MDS**

Myelodysplastic syndrome ("MDS") refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. *The Merck Manual* 953 (17<sup>th</sup> ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424.

The initial hematopoietic stem cell injury can be from causes such as, but not limited to, cytotoxic chemotherapy, radiation, virus, chemical exposure, and genetic predisposition. A clonal mutation predominates over bone marrow, suppressing healthy stem cells. In the early stages of MDS, the main cause of cytopenias is increased programmed cell death (apoptosis). As the disease progresses and converts into leukemia, gene mutation rarely occurs and a proliferation of leukemic cells overwhelms the healthy marrow. The disease course differs, with some cases behaving as an indolent disease and others behaving aggressively with a very short clinical course that converts into an acute form of leukemia.

About 15,000 people are diagnosed with MDS each year in the United States, and about 60,000 are living with the disease. MDS was first considered a distinct disease in 1976, and occurrence was estimated at 1500 new cases every year. At that time, only patients with less than five percent blasts were considered to have this disorder. Statistics from 1999 estimated 13,000 new cases per year and about 1000 cases per

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year in children, surpassing chronic lymphocytic leukemia as the most common form of leukemia in the western hemisphere. The perception that the incidence is increasing may be due to improvements in recognition and criteria for diagnosis.

5 The disease is found worldwide.

An international group of hematologists, the French-American-British (FAB) Cooperative Group, classified MDS disorders into five subgroups, differentiating them from acute myeloid leukemia. *The Merck Manual* 954 (17<sup>th</sup> ed. 1999); Bennett J. M., et al., *Ann. Intern. Med.* 1985 October, 103(4): 620-5; and Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599-617. An underlying trilineage dysplastic change in the bone marrow cells of the patients is found in all subtypes.

There are two subgroups of refractory anemia characterized by five percent or less myeloblasts in bone marrow: (1) refractory anemia (RA) and; (2) RA with ringed sideroblasts (RARS), defined morphologically as having 15% erythroid cells with abnormal ringed sideroblasts, reflecting an abnormal iron accumulation in the mitochondria. Both have a prolonged clinical course and low incidence of progression to acute leukemia. Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599-617.

There are two subgroups of refractory anemias with greater than five percent myeloblasts: (1) RA with excess blasts (RAEB), defined as 6-20% myeloblasts, and (2) RAEB in transformation (RAEB-T), with 21-30% myeloblasts. The higher the percentage of myeloblasts, the shorter the clinical course and the closer the disease is to acute myelogenous leukemia. Patient transition from early to more advanced stages indicates that these subtypes are merely stages of disease rather than distinct entities. Elderly patients with MDS with trilineage dysplasia and greater than 30% myeloblasts who progress to acute leukemia are often considered to have a poor prognosis because their response rate to chemotherapy is lower than de novo acute myeloid leukemia patients. The World Health Organization (WHO) classification (1999) proposes to include all cases of RAEB-T, or patients with greater than 20% myeloblasts, in the category of acute leukemia because these patients have similar prognostic outcomes. However, their response to therapy is worse than the de novo or more typical acute myelogenous leukemia or acute non-lymphocytic leukemia (ANLL) patient. Id.

The fifth type of MDS, the most difficult to classify, is called chronic myelomonocytic leukemia (CMML). This subtype can have any percentage of myeloblasts but presents with a monocytosis of 1000/dL or more. It may be associated with splenomegaly. This subtype overlaps with a myeloproliferative disorder and may have an intermediate clinical course. It is differentiated from the classic chronic myelocytic leukemia (CML) that is characterized by a negative Ph chromosome. The recent WHO classification (1999) proposes that juvenile and proliferative CMML be listed separately from FAB under MDS/myeloproliferative disorder (MPD) with splenomegaly and greater than 13,000 total WBC. CMML is limited to monocytosis, less than 13,000/mm<sup>3</sup> total leukocytes, and requires trilineage dysplasia. Id. Harris N. L., et al., *J. Clin. Oncol.* 1999 December, 17(12): 3835-49. Finally, some other international organizations, including WHO, have suggested a sixth class of MDS patients, characterized by a del (5q) abnormality.

MDS is primarily a disease of elderly people, with the median onset in the seventh decade of life. The median age of these patients is 65 years, with ages ranging from the early third decade of life to as old as 80 years or older. The syndrome may occur in any age group, including the pediatric population. Patients who survive malignancy treatment with alkylating agents, with or without radiotherapy, have a high

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incidence of developing MDS or secondary acute leukemia. About 60-70% of patients do not have an obvious exposure or cause for MDS, and are classified as primary MDS patients.

The most common cases of MDS are primary, or idiopathic. However, a nonspecific history of exposure to indeterminate chemicals or radiation 10-15 years prior to onset of disease may be present in about 50% of patients. This relationship to pathogenesis remains unproved. Compounds such as, but not limited to, benzene, insecticides, weed killers, and fungicides are possible causes of MDS. Goldberg H., et al., *Cancer Res.* 1990 Nov. 1; 50(21): 6876-81. Secondary MDS describes development of MDS or acute leukemia after known exposures to chemotherapy drugs that can cause bone marrow damage. These drugs are associated with a high incidence of chromosomal abnormalities following exposure and at the time of MDS or acute leukemia diagnosis.

Further, MDS is associated with complications associated with severe cytopenias. Other complications are development of myelofibrosis, which can accelerate decline in blood counts and increase transfusion requirements. Transformation to acute leukemia accelerates the development of complications such as anemia, bleeding, and infections.

The International MDS Risk Analysis (IMRA) Workshop proposed an International Prognosis Scoring System (IPSS) to decrease imprecision in predicting survival and AML risk in MDS patients. The IPSS is based on the number of cytopenias, percentage of BM blasts, and type of cytogenetic abnormalities (Table 1). Greenberg et al., *Blood* 1997, 89:2079-88. The latter are categorized into good (normal, -Y, del (5q), del (20q)), intermediate, and poor subgroups (complex or chromosome 7 abnormalities).

TABLE 1

International Prognostic Scoring System for MDS					
	Score Value				
Prognostic Variable	0	0.5	1.0	1.5	2.0
Bone marrow blasts (%)	<5	5-10	—	11-20	21-30
Karyotype*	Good	Intermediate	Poor		
Cytopenias	0/1	2/3			

\*Good, normal, del (5q), del (20q), -Y; Poor, complex (>3) or chromosome 7 abnormalities; Intermediate, +8, and other single or double abnormalities.

### 3.2. MDS Treatment

The current treatment of MDS is based on the stage and the mechanism of the disease that predominates the particular phase of the disease process. Bone marrow transplantation has been used in patients with poor prognosis or late-stage MDS. Epstein and Sleasne, 1985, *Surg. Ann.* 17:125. This type of therapy, however, is both painful for donor and recipient, because of the involvement of invasive procedures and can cause severe and even fatal complications to the recipient, particularly with allogeneic transplant and related Graft Versus Host Disease (GVHD) results. Therefore, the risk of GVHD restricts the use of bone marrow transplantation to patients with otherwise fatal diseases. Further, as most patients are elderly and only a few young MDS patients will have a matched donor, the use of bone marrow transplantation is limited.

An alternative approach to therapy for MDS is the use of hematopoietic growth factors or cytokines to stimulate blood cell development in a recipient. Dexter, 1987, *J. Cell Sci.* 88:1; Moore, 1991, *Annu. Rev. Immunol.* 9:159; and Besa E. C., *Med. Clin. North Am.* 1992 May, 76(3): 599-617. The process of blood cell formation, by which a small number of

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self-renewing stem cells give rise to lineage specific progenitor cells that subsequently undergo proliferation and differentiation to produce the mature circulating blood cells has been shown to be at least in part regulated by specific hormones. These hormones are collectively known as hematopoietic growth factors. Metcalf, 1985, *Science* 229:16; Dexter, 1987, *J. Cell Sci.* 88:1; Golde and Gasson, 1988, *Scientific American*, July: 62; Tabbara and Robinson, 1991, *Anti-Cancer Res.* 11:81; Ogawa, 1989, *Environ. Health Persp.* 80:199; and Dexter, 1989, *Br. Med. Bull.* 45:337. The most well characterized growth factors include erythropoietin (EPO), granulocyte macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF). Apart from inducing proliferation and differentiation of hematopoietic progenitor cells, such cytokines have also been shown to activate a number of functions of mature blood cells, including influencing the migration of mature hematopoietic cells. Stanley et al., 1976, *J. Exp. Med.* 143:631; Schrader et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:323; Moore et al., 1980, *J. Immunol.* 125:1302; Kurland et al., 1979, *Proc. Natl. Acad. Sci. U.S.A.* 76:2326; Handman and Burgess, 1979, *J. Immunol.* 122:1134; Vadas et al., 1983, *Blood* 61:1232; Vadas et al., 1983, *J. Immunol.* 130:795; and Weibart et al., 1986, *J. Immunol.* 137:3584.

Unfortunately, hematopoietic growth factors have not proven effective in many clinical settings. Clinical trials of MDS patients treated with recombinant human GM-CSF and G-CSF have shown that while these cytokines can restore granulocytopenia in treated patients, their efficacy is restricted to the granulocyte or monocyte lineage with little or no improvement in hemoglobin or platelet counts. Schuster et al., 1990, *Blood* 76 (Suppl.1):318a. When such patients were treated with recombinant human EPO, a sustained improvement in hemoglobin or decrease in transfusion requirement was achieved in only less than 25% of patients. Besa et al., 1990, 76 (Suppl.1):133a; Hellstrom et al., 1990, 76 (Suppl.1): 279a; Bowen et al., 1991, *Br. J. Haematol.* 77:419. Therefore, there remains a need for safe and effective methods of treating and managing MDS.

### 3.3. Thalidomide and Azacitidine

Thalidomide is a racemic compound sold under the trade-name Thalomid® and chemically named  $\alpha$ -(N-phthalimido) glutarimide or 2-(2,6-dioxo-3-piperidinyl)-1H-isoindeole-1,3 (2H)-dione. Thalidomide was originally developed in the 1950's to treat morning sickness, but due to its teratogenic effects was withdrawn from use. Thalidomide has been approved in the United States for the acute treatment of the cutaneous manifestations of erythema nodosum leprosum in leprosy. *Physicians' Desk Reference*, 1154-1158 (56<sup>th</sup> ed., 2002). Because its administration to pregnant women can cause birth defects, the sale of thalidomide is strictly controlled. Id. Thalidomide has reportedly been studied in the treatment of other diseases, such as chronic graft-vs-host disease, rheumatoid arthritis, sarcoidosis, several inflammatory skin diseases, and inflammatory bowel disease. See generally, Koch, H. P., *Prog. Med. Chem.* 22:165-242 (1985). See also, Moller, D. R., et al., *J. Immunol.* 159:5157-5161 (1997); Vasiliauskas, E. A., et al., *Gastroenterology* 117:1278-1287 (1999); Ehrenpreis, E. D., et al., *Gastroenterology* 117:1271-1277 (1999). It has further been alleged that thalidomide can be combined with other drugs to treat ischemia/reperfusion associated with coronary and cerebral occlusion. See U.S. Pat. No. 5,643,915, which is incorporated herein by reference.

More recently, thalidomide was found to exert immunomodulatory and anti-inflammatory effects in a variety of disease states, cachexia in AIDS, and opportunistic infections in



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AIDS. In studies to define the physiological targets of thalidomide, the drug was found to have a wide variety of biological activities exclusive of its sedative effect including neurotoxicity, teratogenicity, suppression of TNF- $\alpha$  production by monocytes/macrophages and the accompanying inflammatory toxicities associated with high levels of TNF- $\alpha$ , and inhibition of angiogenesis and neovascularization.

Additionally, beneficial effects have been observed in a variety of dermatological conditions, ulcerative colitis, Crohn's disease, Bechets's syndrome, systemic lupus erythematosus, aphthous ulcers, and lupus. The anti-angiogenic properties of thalidomide in in vivo models have been reported. D'Amato et al., *Thalidomide Is An Inhibitor Of Angiogenesis*, 1994, *PNAS, USA* 91:4082-4085.

One of the most therapeutically significant potential uses of thalidomide is in the treatment of cancer. The compound has been investigated in the treatment of various types of cancer, such as refractory multiple myeloma, brain, breast, colon, and prostate cancer, melanoma, mesothelioma, and renal cell carcinoma. See, e.g., Singhal, S., et al., *New England J. Med.* 341(21):1565-1571 (1999); and Marx, G. M., et al., *Proc. Am. Soc. Clin. Oncology* 18:454a (1999). Thalidomide reportedly can also be used to prevent the development of chronic cardiomyopathy in rats caused by doxorubicin. Costa, P. T., et al., *Blood* 92(10:suppl. 1):235b (1998). Other reports concerning the use of thalidomide in the treatment of specific cancers include its combination with carboplatin in the treatment of glioblastoma multiforme. McCann, J., *Drug Topics* 41-42 (Jun. 21, 1999). The use of thalidomide in combination with dexamethasone reportedly was effective in the treatment of patients suffering from multiple myeloma who also received, as supportive care, human granulocyte colony-stimulating factor (G-CSF), ciprofloxacin, and non-absorbable antifungal agents. Kropff, M. H., *Blood* 96(11 part 1):168a (2000); see also, Munshi, N. et al., *Blood* 94(10 part 1):578a (1999). Other chemotherapy combinations that comprise thalidomide are disclosed in International Application No. PCT/US01/15326 to R. Govindarajan and A. Zeitlan, and in International Application No. PCT/US01/15327 to J. B. Zeldis, et al.

In an effort to provide compounds that have greater therapeutic safety and efficacy than thalidomide, researchers have begun investigating a large number of other compounds, some of which are derivatives of thalidomide. See, e.g., Marriott, J. B., et al., *Expert Opin. Biol. Ther.* 1(4):1-8 (2001); G. W. Muller, et al., *Journal of Medicinal Chemistry* 39(17):3238-3240 (1996); and G. W. Muller, et al., *Bioorganic & Medicinal Chemistry Letters* 8:2669-2674 (1998). Examples include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles described in U.S. Pat. Nos. 6,281,230 and 6,316,471, both to G. W. Muller, et al.

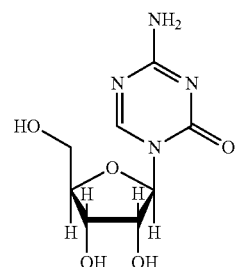
A group of compounds selected for their capacity to potentially inhibit TNF- $\alpha$  production by LPS stimulated PBMC has been investigated. L. G. Corral, et al., *Ann. Rheum. Dis.* 58:(Suppl I) 1107-1113 (1999). These compounds, which are referred to as IMiDs™ or Immunomodulatory Drugs, show not only potent inhibition of TNF- $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  and IL12 production. LPS induced IL6 is also inhibited by IMiDs™, albeit partially. These compounds are potent stimulators of LPS induced IL10, increasing IL10 levels by 200 to 300%. Id.

In addition, nucleoside analogs have been used clinically for the treatment of viral infections and proliferative disorders for decades. Most of the nucleoside analog drugs are classified as antimetabolites. After they enter cells, nucleoside analogs are successively phosphorylated to nucleoside 5'-mono-

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phosphates, 5'-diphosphates, and 5'-triphosphates. In most cases, nucleoside triphosphates are the chemical entities that inhibit DNA or RNA synthesis, either through a competitive inhibition of polymerases or through incorporation of modified nucleotides into DNA or RNA sequences. Nucleosides may act also as their diphosphates.

5-Azacytidine (also known as azacitidine and 4-amino-1- $\beta$ -D-ribofuranosyl-1,3,5-triazin-2(1H)-one; Nacion Service Center designation NSC-102816; CAS Registry Number 320-67-2) has undergone NCI-sponsored trials for the treatment of MDS. See, e.g., Kornblith et al., *J. Clin. Oncol.* 20(10):2441-2452 (2002); Silverman et al., *J. Clin. Oncol.* 20(10):2429-2440 (2002). 5-Azacytidine may be defined as having a molecular formula of C<sub>8</sub>H<sub>12</sub>N<sub>4</sub>O<sub>5</sub>, a relative molecular weight of 244.21 and a structure of:



Azacitidine (also referred to as 5-azacytidine herein) is a nucleoside analog, more specifically a cytidine analog. 5-Azacytidine is an antagonist of its related natural nucleoside, cytidine. 5-Azacytidine, as well as decitabine, i.e., 5-aza-2'-deoxycytidine, are antagonists of decitabine's related natural nucleoside, deoxycytidine. The only structural difference between the analogs and their related natural nucleosides is the presence of nitrogen at position 5 of the cytosine ring in place of oxygen.

Other members of the class of deoxycytidine and cytidine analogs include arabinosylcytosine (Cytarabine), 2'-deoxy-2',2'-difluorocytidine (Gemcitabine), 5-aza-2'-deoxycytidine (Decitabine), 2(1H)-pyrimidine-ribose (Zebularine), 2',3'-dideoxy-5-fluoro-3'-thiacytidine (Emtriva), N<sup>4</sup>-pentyl-oxycarbonyl-5'-deoxy-5-fluorocytidine (Capecitabine), 2'-cyclocytidine, arabinofuranosyl-5-azacytidine, dihydro-5-azacytidine, N<sup>4</sup>-octadecyl-cytarabine, elaidic acid cytarabine, and cytosine 1- $\beta$ -D-arabinofuranoside (ara-C).

There remains a need for more effective methods for treating MDS and its related disorders.

#### 4. SUMMARY

Embodiments herein provide methods for the treatment of myelodysplastic syndromes (MDS) with a combination therapy using a therapeutically effective amount of a cytidine analog, including, but not limited to, 5-azacytidine, and a therapeutically effective amount of an immunomodulatory compound, including, but not limited to, 3-(4-amino-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (lenalidomide) or a pharmaceutically acceptable salt, solvate (e.g., hydrate) or stereoisomer thereof. Particular embodiments provide methods for treating patients with advanced MDS or higher risk MDS using the combination therapy. Particular embodiments provide methods for improving the overall survival of patients having MDS, e.g., higher risk MDS. Particular embodiments provide alternative dosing regimens for treating MDS. Particular embodiments provide methods for

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treating patients having MDS, using specific numbers of lenalidomide and azacitidine treatment cycles. Particular embodiments provide methods of treating patients who meet the WHO criteria for AML using lenalidomide and azacitidine. Particular embodiments provide using lenalidomide and azacitidine as maintenance therapy.

One embodiment encompasses the use of lenalidomide and azacitidine in combination with conventional therapies presently used to treat, prevent or manage MDS such as hematopoietic growth factors, cytokines, cancer chemotherapeutics, stem cell transplantation and other transplantations.

## 5. DETAILED DESCRIPTION

A first embodiment encompasses methods of treating or preventing MDS which comprise administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of 3-(4-amino-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (lenalidomide), or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with a therapeutically or prophylactically effective amount of a cytidine analog, particularly, 5-azacytidine. The embodiment encompasses the treatment, prevention or management of specific sub-types of MDS such as refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia.

As used herein, the term “myelodysplastic syndromes” or “MDS” means hematopoietic stem cell disorders characterized by one or more of the following: ineffective blood cell production, progressive cytopenias, risk of progression to acute leukemia or cellular marrow with impaired morphology and maturation (dysmyelopoiesis). The term “myelodysplastic syndromes” or “MDS” unless otherwise noted includes: refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation and chronic myelomonocytic leukemia.

Another embodiment encompasses methods of managing MDS which comprises administering to a patient in need of such management a prophylactically effective amount of lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with a therapeutically or prophylactically effective amount of 5-azacytidine.

Another embodiment encompasses a kit comprising: a pharmaceutical composition comprising (1) lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and (2) 5-azacytidine, and/or (3) instructions for use. The invention further encompasses kits comprising single unit dosage forms.

One embodiment encompasses a method of treating, preventing and/or managing MDS, which comprises administering to a patient in need of such treatment, prevention and/or management therapeutically or prophylactically effective amounts of 5-azacytidine and lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a therapeutically or prophylactically effective amount of an additional active agent.

The additional active agent is preferably a hematopoietic growth factor, a cytokine, an anti-cancer agent, an antibiotic, an anti-fungal, an anti-inflammatory, an immunosuppressive agent such as a cyclosporin, conventional therapy for MDS, or other chemotherapeutic agent found for example in the Physician's Desk Reference. Preferred anti-cancer or cancer chemotherapeutics are apoptosis inducing agents, topoi-

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somerase inhibitors, anti-angiogenesis compounds, microtubule stabilizing agents, alkylating agents and other known conventional cancer chemotherapy. Most preferred additional active agents are those capable of affecting or improving blood production. The active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules). The examples of specific additional active agent include, but are not limited to, gemtuzumab ozogamicin, etanercept (Enbrel®), imatinib (Glivec®), anti-TNF- $\alpha$  antibodies, infliximab (Remicade®), G-CSF, GM-CSF, EPO, topotecan, irinotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, vinblastine, isotretinoin, and 13-cis-retinoic acid. This invention also encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term “mutants” are proteins that lack carbohydrate moieties normally present in their naturally occurring forms (e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001). Vaccines that cause the secretion of proteins disclosed herein as well as pharmacologically active mutants, derivatives, and fusion thereof are also encompassed by the invention.

Without being limited by theory, it is believed that certain immunomodulatory compounds and proteins can act in complementary or synergistic ways in the treatment or management of MDS. It is also believed that certain proteins may reduce or eliminate particular adverse effects associated with some immunomodulatory compounds, thereby allowing the administration of larger amounts of an immunomodulatory compound to patients and/or increasing patient compliance. It is further believed that some immunomodulatory compounds may reduce or eliminate particular adverse effects associated with some protein-based MDS therapies, thereby allowing the administration of larger amounts of protein to patients and/or increasing patient compliance.

Another embodiment of the invention encompasses a method of reversing, reducing or avoiding an adverse effect associated with the administration of a chemotherapeutics or therapeutics used to treat cancer or MDS in a patient suffering from MDS, which comprises administering to a patient in need thereof therapeutically or prophylactically effective amounts of azacitidine and lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

As inevitable leukemic transformation develops in certain stages of MDS, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. It is believed that the combined use of an immunomodulatory compound and transplantation of stem cells in a patient suffering from MDS provides a unique and unexpected synergism. In particular, without being limited by theory, it is believed that an immunomodulatory compound exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy. Immunomodulatory compounds can work in combination with transplantation therapy reducing com-



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plications associated with the invasive procedure of transplantation and risk of related Graft Versus Host Disease (GVHD). Therefore, this invention encompasses a method of treating, preventing and/or managing MDS, which comprises administering to a patient (e.g., a human) azacitidine and lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after transplantation therapy.

The invention also encompasses pharmaceutical compositions, single unit dosage forms, and kits which comprise azacitidine and lenalidomide, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, an additional active ingredient, and/or blood or cells for transplantation therapy. For example, the kit may contain one or more compounds of the invention, stem cells for transplantation and an immunosuppressive agent, antibiotic or other drug, each of which is to be used to treat the MDS patient.

#### 5.1. Immunomodulatory Compounds

Compounds used in the invention include immunomodulatory compounds that are racemic, stereomerically enriched or stereomerically pure, and pharmaceutically acceptable salts, solvates, hydrates, stereoisomers, clathrates, and prodrugs thereof. Preferred compounds used in the invention are small organic molecules having a molecular weight less than about 1000 g/mol, and are not proteins, peptides, oligonucleotides, oligosaccharides or other macromolecules.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. As used herein and unless otherwise indicated, the term "stereomerically enriched" means a composition that comprises greater than about 60% by weight of one stereoisomer of a compound, preferably greater than about 70% by weight, more preferably greater than about 80% by weight of one stereoisomer of a compound. As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center. Similarly, the term "stereomerically enriched" means a stereomerically enriched composition of a compound having one chiral center.

As used herein and unless otherwise indicated, the term "immunomodulatory compounds" or "IMiDs™" (Celgene Corporation) used herein encompasses small organic molecules that markedly inhibit TNF- $\alpha$ , LPS induced monocyte IL1 $\beta$  and IL12, and partially inhibit IL6 production. Specific immunomodulatory compounds of the invention are discussed below.

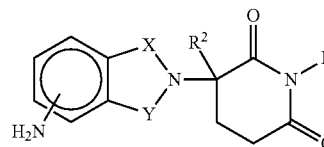
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TNF- $\alpha$  is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation. TNF- $\alpha$  is responsible for a diverse range of signaling events within cells. TNF- $\alpha$  may play a pathological role in cancer. Without being limited by particular theory, one of the biological effects exerted by the immunomodulatory compounds of the invention is the reduction of synthesis of TNF- $\alpha$ . Immunomodulatory compounds of the invention enhance the degradation of TNF- $\alpha$  mRNA.

Further, without being limited by particular theory, immunomodulatory compounds used in the invention may also be potent co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. Immunomodulatory compounds of the invention may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset. In addition, the compounds preferably have anti-inflammatory properties, and efficiently co-stimulate T cells.

Specific examples of immunomodulatory compounds of the invention, include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl) isoindolines such as those described in U.S. Pat. No. 5,874,448; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolines described in U.S. Pat. No. 5,798,368; t-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines (e.g., 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517; and a class of non-polypeptide cyclic amides disclosed in U.S. Pat. Nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S. Pat. Nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles such as those described in U.S. Pat. Nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those described in U.S. patent application Ser. No. 09/972,487 filed on Oct. 5, 2001, U.S. patent application Ser. No. 10/032,286 filed on Dec. 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents identified herein are incorporated herein by reference. Immunomodulatory compounds of the invention do not include thalidomide.

Other specific immunomodulatory compounds of the invention include, but are not limited to, 1-oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein. These compounds have the structure I:



I

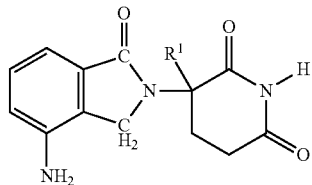
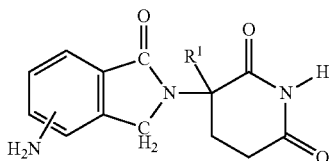
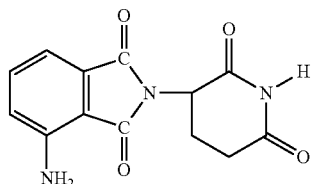
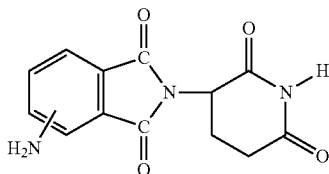
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in which one of X and Y is C=O, the other of X and Y is C=O or CH<sub>2</sub>, and R<sup>2</sup> is hydrogen or lower alkyl, in particular methyl. Specific immunomodulatory compounds include, but are not limited to:

1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;  
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

Other specific immunomodulatory compounds of the invention belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles, such as those described in U.S. Pat. Nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (International Publication No. WO 98/03502), each of which is incorporated herein. Compounds representative of this class are of the formulas:

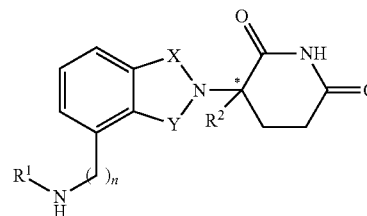


wherein R<sup>1</sup> is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (e.g. optically pure (R) or (S) enantiomers) of these compounds.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. Nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are

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incorporated herein by reference. Representative compounds are of formula II:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

R<sup>1</sup> is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, C(O)R<sup>3</sup>, C(S)R<sup>3</sup>, C(O)OR<sup>4</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, C(O)NHR<sup>3</sup>, C(S)NHR<sup>3</sup>, C(O)NR<sup>3</sup>R<sup>3'</sup>, C(S)NR<sup>3</sup>R<sup>3'</sup> or (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>;

R<sup>2</sup> is H, F, benzyl, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, or (C<sub>2</sub>-C<sub>8</sub>)alkynyl;

R<sup>3</sup> and R<sup>3'</sup> are independently (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>0</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>, or C(O)OR<sup>5</sup>;

R<sup>4</sup> is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, (C<sub>1</sub>-C<sub>4</sub>)alkyl-OR<sup>5</sup>, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, or (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl;

R<sup>5</sup> is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, or (C<sub>2</sub>-C<sub>5</sub>)heteroaryl;

each occurrence of R<sup>6</sup> is independently H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>2</sub>-C<sub>5</sub>)heteroaryl, or (C<sub>0</sub>-C<sub>8</sub>)alkyl-C(O)O—R<sup>5</sup> or the R<sup>6</sup> groups can join to form a heterocycloalkyl group;

n is 0 or 1; and

\* represents a chiral-carbon center.

In specific compounds of formula II, when n is 0 then R<sup>1</sup> is (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, C(O)R<sup>3</sup>, C(O)OR<sup>4</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, C(S)NHR<sup>3</sup>, or (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>;

R<sup>2</sup> is H or (C<sub>1</sub>-C<sub>8</sub>)alkyl; and

R<sup>3</sup> is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>5</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>; (C<sub>0</sub>-C<sub>8</sub>)alkyl-NH—C(O)O—R<sup>5</sup>; (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>, or C(O)OR<sup>5</sup>; and the other variables have the same definitions.

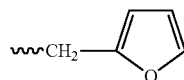
In other specific compounds of formula II, R<sup>2</sup> is H or (C<sub>1</sub>-C<sub>4</sub>)alkyl.

In other specific compounds of formula II, R<sup>1</sup> is (C<sub>1</sub>-C<sub>8</sub>)alkyl or benzyl.

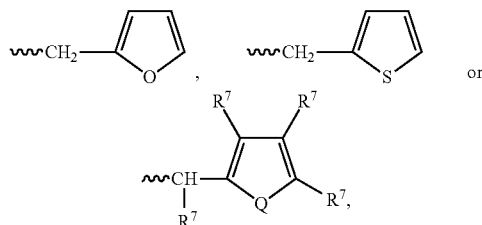
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In other specific compounds of formula II, R<sup>1</sup> is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, benzyl, CH<sub>2</sub>OCH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, or



In another embodiment of the compounds of formula II, R<sup>1</sup> is



wherein Q is O or S, and each occurrence of R<sup>7</sup> is independently H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, benzyl, CH<sub>2</sub>OCH<sub>3</sub>, or CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>.

In other specific compounds of formula II, R<sup>1</sup> is C(O)R<sup>3</sup>.

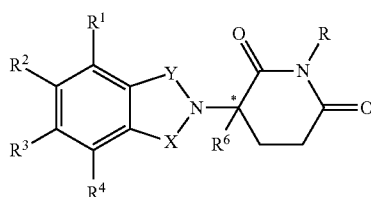
In other specific compounds of formula II, R<sup>3</sup> is (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>1</sub>-C<sub>8</sub>)alkyl, aryl, or (C<sub>0</sub>-C<sub>4</sub>)alkyl-OR<sup>5</sup>.

In other specific compounds of formula II, heteroaryl is pyridyl, furyl, or thienyl.

In other specific compounds of formula II, R<sup>1</sup> is C(O)OR<sup>4</sup>.

In other specific compounds of formula II, the H of C(O)NHC(O) can be replaced with (C<sub>1</sub>-C<sub>4</sub>)alkyl, aryl, or benzyl.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. No. 09/781,179, International Publication No. WO 98/54170, and U.S. Pat. No. 6,395,754, each of which are incorporated herein by reference. Representative compounds are of formula III:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

R is H or CH<sub>2</sub>OCOR<sup>5</sup>;

(i) each of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup>, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> is nitro or —NHR<sup>5</sup> and the remaining of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> are hydrogen;

R<sup>5</sup> is hydrogen or alkyl of 1 to 8 carbons;

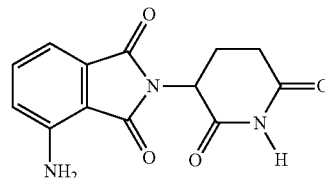
R<sup>6</sup> hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro; and

\* represents a chiral-carbon center.

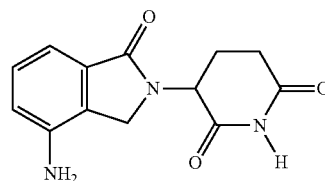
The most preferred immunomodulatory compounds of the invention are 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindol-

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line-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. The compounds can be obtained via standard, synthetic methods (see e.g., U.S. Pat. No. 5,635, 517, incorporated herein by reference). The compounds are available from Celgene Corporation, Summit, N.J. 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (pomalidomide) has the following chemical structure:



3-(4 Amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (lenalidomide) has the following chemical structure:



The compounds of the invention can either be commercially purchased or prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry techniques.

As used herein and unless otherwise indicated, the term “pharmaceutically acceptable salt” encompasses non-toxic acid and base addition salts of the compound to which the term refers. Acceptable non-toxic acid addition salts include those derived from organic and inorganic acids or bases known in the art, which include, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embolic acid, enanthic acid, and the like.

Compounds that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds are those that form non-toxic base addition salts, i.e., salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), lysine, and procaine.

As used herein and unless otherwise indicated, the term “prodrug” means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of immunomodulatory compounds of the invention that comprise bio-

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hydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of immunomodulatory compounds of the invention that comprise —NO, —NO<sub>2</sub>, —ONO, or —ONO<sub>2</sub> moieties. Prodrugs can typically be prepared using well known methods, such as those described in 1 *Burger's Medicinal Chemistry and Drug Discovery*, 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995), and *Design of Prodrugs* (H. Bundgaard ed., Elsevier, N.Y. 1985).

As used herein and unless otherwise indicated, the terms “biohydrolyzable amide,” “biohydrolyzable ester,” “biohydrolyzable carbamate,” “biohydrolyzable carbonate,” “biohydrolyzable ureide,” “biohydrolyzable phosphate” mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxyethyl, acetoxyethyl, aminocarbonyloxyethyl, pivaloyloxyethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyloxyethyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides,  $\alpha$ -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

#### 5.2. Additional Active Agents

One or more additional active ingredients can be used in the methods and compositions of the invention together with azacytidine and an immunomodulatory compound of the invention. In a preferred embodiment, the additional active agents are capable of affecting or improving the process of blood cell production. Specific additional active agents also stimulate the division and differentiation of committed erythroid progenitors in cells in vitro or in vivo.

Additional active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules). The additional active agents include but are not limited to hematopoietic growth factors, cytokines, anti-cancer agents, antibiotics, proteasome inhibitors, immunosuppressive agents and other therapeutics discussed herein. Particular agents include, but are not limited to, G-CSF, GM-CSF, EPO, dexamethasone, topotecan, pentoxifylline, irinotecan, ciprofloxacin, vinorelbine, IL2, IL8, IL18, Ara-C, isotretinoin, 13-cis-retinoic acid, 12-O-tetradecanoylphorbol-13-acetate (TPA), 5-AZA2'-deoxycytidine, 9-nitrocamp-tothecin, transretinoic acid, amifostine, amphotericin B and liposomal amphotericin B, anti-CD-20 monoclonal antibody, anti-thymocyte globulin (ATG), arsenic tri-

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oxide, azacytidine, bevacizumab, bismuth monoclonal antibody, bryostatin, busulfan, caspofungin acetate, celastrol, cladribine, cyclophosphamide, cyclosporine, cytarabine, cytosine, daunorubicin, desisoleptide, etoposide, farfesy transferase inhibitor, flavopiridol, Flt3 ligand, fludarabine, gentuzumab ozogomicin (mylotarg), etanercept (Enbrel®), imatinib (Glivec®), anti-TNF- $\alpha$  antibodies, infliximab (Remicade®), humanized monoclonal anti-VEGF antibody, idarubicin, leucovorin, melphalan, mitoxantrone, monoclonal antibody ABX-CBL, monoclonal antibody CD52, mycophenolate mofetil, oblimersen, omega-3 fatty acids, pentostatin, phenylbutyrate, PR1 leukemia peptide vaccine, montanide, proteasome inhibitor, sodium phenylbutyrate, sodium salicylate, temozolomide, thymoglobulin, troxatyl, tumor necrosis factor receptor IgG chimera, Yttrium Y 90 humanized monoclonal antibody M195. In a specific embodiment of the invention, an immunomodulatory compound of the invention is used in combination with pentoxifylline, ciprofloxacin, and/or dexamethasone.

This invention also encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term “mutants” are proteins that lack carbohydrate moieties normally present in their naturally occurring forms (e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001).

Recombinant and mutated forms of G-CSF can be prepared as described in U.S. Pat. Nos. 4,810,643; 4,999,291; 5,528,823; and 5,580,755; all of which are incorporated herein by reference. Recombinant and mutated forms of GM-CSF can be prepared as described in U.S. Pat. Nos. 5,391,485; 5,393,870; and 5,229,496; all of which are incorporated herein by reference. In fact, recombinant forms of G-CSF and GM-CSF are currently sold in the United States for the treatment of symptoms associated with specific chemotherapies. A recombinant form of G-CSF known as filgrastim is sold in the United States under the trade name NEUPOGEN®. NEUPOGEN® is known to stimulate division and maturation of granulocytes, mostly neutrophils, in MDS patients and to enhance erythroid response in combination with EPO. *Physicians' Desk Reference*, 587-592 (56<sup>th</sup> ed., 2002). A recombinant form of GM-CSF known as sargramostim is also sold in the United States under the trade name LEUKINE®. LEUKINE® is known to stimulate division and maturation of earlier myeloid and macrophage precursor cells and has been reported to increase granulocytes. *Physicians' Desk Reference*, 1755-1760 (56<sup>th</sup> ed., 2002). A recombinant form of EPO known as epoetin alfa is sold in the United States under the trade name EPOGEN®. EPOGEN® is used to stimulate red cell production by stimulating division and maturation of committed red cell precursor cells. EPOGEN® has been reported to be effective in 20-26% of MDS patient when administered by itself and in as many as 48% of patients when combined with G-CSF or GM-CSF. *Physicians' Desk Reference*, 582-587 (56<sup>th</sup> ed., 2002).

A growth-factor or cytokine such as G-CSF, GM-CSF and EPO can also be administered in the form of a vaccine. For



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example, vaccines that secrete, or cause the secretion of, cytokines such as G-CSF and GM-CSF can be used in the methods, pharmaceutical compositions, and kits of the invention. See, e.g., Emens, L. A., et al., *Curr. Opinion Mol. Ther.* 3(1):77-84 (2001).

Other compounds that can be administered or used in combination with an immunomodulatory compound of the invention include those disclosed in U.S. provisional patent application No. 60/380,842, filed May 17, 2002, and U.S. provisional patent application No. 60/380,843, filed May 17, 2002, both of which are incorporated herein by reference.

### 5.3. Methods of Treatment and Management

Methods of this invention encompass methods of preventing, treating and/or managing various types of MDS. As used herein, unless otherwise specified, the term "preventing" includes but is not limited to, inhibition or the averting of symptoms associated with MDS. The symptoms associated with MDS include, but are not limited to, anemia, thrombocytopenia, neutropenia, cytopenia, bicytopenia (two deficient cell lines), and pancytopenia (three deficient cell lines). As used herein, unless otherwise specified, the term "treating" refers to the administration of a composition after the onset of symptoms of MDS, whereas "preventing" refers to the administration prior to the onset of symptoms, particularly to patients at risk of MDS. As used herein and unless otherwise indicated, the term "managing" encompasses preventing the recurrence of MDS in a patient who had suffered from MDS, lengthening the time a patient who had suffered from MDS remains in remission, and/or preventing the occurrence of MDS in patients at risk of suffering from MDS.

The invention encompasses methods of treating or preventing patients with primary and secondary MDS. It further encompasses methods treating patients who have been previously treated for MDS, as well as those who have not previously been treated for MDS. Because patients with MDS have heterogenous clinical manifestations and varying clinical outcomes, it has become apparent that staging the patients according to their prognosis and approaching therapy depending on the severity and stage is necessary. Indeed, the methods and compositions of this invention can be used in various stages of treatments for patients with one or more types of MDS including, but not limited to, refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess blasts (RAEB), RAEB in transformation (RAEB-T), or chronic myelomonocytic leukemia (CMML). The invention also contemplates treating patients diagnosed using the IPSS for MDS discussed above. Greenberg et al., *Blood* 1997 (89): 2079-88.

Methods encompassed by this invention comprise administering azacitidine and an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof to a patient (e.g., a human) suffering, or likely to suffer, from MDS. Specific patient populations include the elderly, i.e., ages 60 and above as well as those over 35 years of age. Patients with familial history of MDS or leukemia are also preferred candidates for preventive regimens.

In one embodiment of the invention, an immunomodulatory compound of the invention is administered orally and in a single or divided daily doses in an amount of from about 0.10 to about 150 mg/day. In a particular embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (pomalidomide) is administered in an amount of from about 0.1 to about 10 mg per day, or alternatively every other day. 3-(4-Amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,

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6-dione (lenalidomide) can be preferably administered in an amount of from about 5 to 25 mg per day, or alternatively every other day.

In one embodiment of the invention, azacitidine is administered according to one of three alternative dosing schedules, administered in 28-day cycles:

- 1) AZA 5-2-2: azacitidine 75 mg/m<sup>2</sup>/day SC×5 days, followed by 2 days of no treatment, followed by azacitidine 75 mg/m<sup>2</sup>/day SC×2 days;
- 2) AZA 5-2-5: azacitidine 50 mg/m<sup>2</sup>/day SC×5 days, followed by 2 days of no treatment, followed by azacitidine 50 mg/m<sup>2</sup>/day SC×5 days; and
- 3) AZA 5: azacitidine 75 mg/m<sup>2</sup>/day SC×5 days.

After at least 2 cycles, azacitidine dose can be increased if the patient is not responding, defined as treatment failure or disease progression according to IWG 2000 criteria for MDS (≥50% increase in blasts, ≥50% decrease from maximum response levels in granulocytes or platelets, hemoglobin reduction ≥2 g/dL, or transfusion independence). Conversely, the dose can be decreased based on hematological recovery and adverse events.

### 5.3.1 Combination Therapy with Additional Active Agent

Particular methods of the invention comprise administering 1) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, 2) azacitidine and 3) an additional active agent or active ingredient. Examples of immunomodulatory compounds of the invention are disclosed herein (see, e.g., section 5.1); and examples of the additional active agents are also disclosed herein (see, e.g., section 5.2).

Administration of the immunomodulatory compounds, azacitidine and the additional active agents to a patient can occur simultaneously or sequentially by the same or different routes of administration. The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. A preferred route of administration for an immunomodulatory compound is oral. A preferred route of administration for azacitidine is subcutaneous, intravenous or oral. Preferred routes of administration for the additional active agents or ingredients of the invention are known to those of ordinary skill in the art. See, e.g., *Physicians' Desk Reference*.

In one embodiment, the additional active agent is administered intravenously or subcutaneously and once or twice daily in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. The specific amount of the additional active agent will depend on the specific agent used, the type of MDS being treated or managed, the severity and stage of MDS, and the amount(s) of immunomodulatory compounds of the invention and any optional additional active agents concurrently administered to the patient. In a particular embodiment, the additional active agent is gemtuzumab ozogamicin, etanercept, GM-CSF, G-CSF, EPO, transretinoic acid, dexamethasone, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, or a combination thereof. GM-CSF is administered in an amount of from about 60 to about 500 mcg/m<sup>2</sup> intravenously over 2 hours, or from about 5 to about 12 mcg/m<sup>2</sup>/day subcutaneously. G-CSF is administered subcutaneously in an amount of about 1 mcg/kg/day initially and can be adjusted depending on rise of total granulocyte counts. The maintenance dose is 300 (in smaller patients) or 480 mcg

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subcutaneously. EPO is administered subcutaneously in an amount of 10,000 Unit 3 times per week.

#### 5.3.2 Use with Transplantation Therapy

In still another embodiment, this invention encompasses a method of treating, preventing and/or managing MDS, which comprises administering azacitidine and the immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with transplantation therapy. As discussed elsewhere herein, the treatment of MDS is based on the stages and mechanism of the disease. As inevitable leukemic transformation develops in certain stages of MDS, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. The combined use of azacitidine, the immunomodulatory compound of the invention and transplantation therapy provides a unique and unexpected synergism. In particular, an immunomodulatory compound of the invention exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy in patients with MDS. An immunomodulatory compound of the invention can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of related Graft Versus Host Disease (GVHD). This invention encompasses a method of treating, preventing and/or managing MDS which comprises administering to a patient (e.g., a human) azacitidine and an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after the transplantation of umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow. Examples of stem cells suitable for use in the methods of the invention are disclosed in U.S. provisional patent application No. 60/372,348, filed Apr. 12, 2002 by R. Hariri et al., the entirety of which is incorporated herein by reference.

#### 5.3.3 Cycling Therapy

In a preferred embodiment, the therapeutic agents of the invention are cyclically administered to a patient. Cycling therapy involves the administrations of the first agent and the second agent for periods of time, followed by rest for periods of time, and repeating the sequential administrations. Cycling therapy can reduce the development of resistance to the therapies, avoid or reduce the side effects of the therapies, and/or improves the efficacy of the treatment.

In a particular embodiment, one cycle can comprise the administration of a therapeutic agent for three (3) weeks followed by one (1) week of rest in a 28-days cycle. The number of cycles administered is from about 1 to about 12 cycles. 3-(4-Amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidin-2,6-dione (lenalidomide) is orally administered to patients with MDS in an amount of about 5-25 mg per day for 21 days followed by seven days rest on a 28 day cycle. In a particular embodiment, lenalidomide is orally administered to patients with MDS in an amount of about 10 mg per day for 21 days followed by seven days rest on a 28 day cycle.

In a particular embodiment, the patient also receive 5-azacytidine at a dose of about 25-75 mg/m<sup>2</sup>/d subcutaneously (SC) or intravenously (IV) for days 1-7 followed by twenty-one days rest on a 28 day cycle. In another particular embodiment, patient receive 5-azacytidine at a dose of about 25-75 mg/m<sup>2</sup>/d subcutaneously (SC) or intravenously (IV) for days 1-5 followed by twenty-three days rest on a 28 day cycle. In another particular embodiment, patient receive oral 5-azacytidine at a dose of about 120 mg/d for days 1-7 followed by twenty-one days rest on a 28 day cycle.

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In one embodiment, up to 9 or more 28-day cycles are administered. Other methods for providing an effective amount of a cytidine analog are disclosed in, for example, "Colon-Targeted Oral Formulations of Cytidine Analogs", U.S. Ser. No. 11/849,958, which is incorporated by reference herein in its entirety.

#### 5.4. Pharmaceutical Compositions and Single Unit Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Pharmaceutical compositions and dosage forms of the invention can further comprise one or more excipients.

Pharmaceutical compositions and dosage forms of the invention can also comprise one or more additional active ingredients. Consequently, pharmaceutical compositions and dosage forms of the invention comprise the active ingredients disclosed herein (e.g., an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and an additional active ingredient). Examples of optional additional active ingredients are disclosed herein (see, e.g., section 5.2).

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), or parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), transdermal or transcutaneous administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18<sup>th</sup> ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage



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form. For example, the decomposition of some active ingredients may be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose other mono- or disaccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the *U.S. Pharmacopeia* (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of from about 0.10 to about 150 mg. Typical dosage forms comprise an immuno-

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modulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of about 0.1, 1, 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 50, 100, 150 or 200 mg. In a particular embodiment, a preferred dosage form comprises 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) in an amount of about 1, 2, 5, 10, 25 or 50 mg. In a specific embodiment, a preferred dosage form comprises 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine 2,6-dione (Lenalidomide) in an amount of about 5, 10, 25 or 50 mg.

Pharmaceutical compositions may contain sufficient quantities of azacitidine to provide a daily dosage of about 10 to 150 mg/m<sup>2</sup> (based on patient body surface area) or about 0.1 to 4 mg/kg (based on patient body weight) as single or divided (2-3) daily doses. In one embodiment, dosage of azacitidine is provided via a seven day administration of 75 mg/m<sup>2</sup> subcutaneously, once every twenty-eight days, for as long as clinically necessary.

Typical dosage forms comprise the additional active ingredient in an amount of 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. Of course, the specific amount of the additional active ingredient will depend on the specific agent used, the type of MDS being treated or managed, and the amount(s) of immunomodulatory compounds and azacitidine, and any optional additional active agents concurrently administered to the patient.

#### 5.4.1 Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences*, 18<sup>th</sup> ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in

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a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. An specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a

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pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

A preferred solid oral dosage form of the invention comprises an immunomodulatory compound of the invention, anhydrous lactose, microcrystalline cellulose, polyvinylpyrrolidone, stearic acid, colloidal anhydrous silica, and gelatin.

#### 5.4.2 Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydroxypropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

#### 5.4.3 Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or

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suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention. For example, cyclodextrin and its derivatives can be used to increase the solubility of an immunomodulatory compound of the invention, and its derivatives. See, e.g., U.S. Pat. No. 5,134,127, which is incorporated herein by reference.

#### 5.4.4 Topical and Mucosal Dosage Forms

Topical and mucosal dosage forms of the invention include, but are not limited to, sprays, aerosols, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide topical and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form solutions, emulsions or gels, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990).

The pH of a pharmaceutical composition or dosage form may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

#### 5.4.5 Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

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A typical kit of the invention comprises a dosage form of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, pro-drug, or clathrate thereof. Kits encompassed by this invention can further comprise additional active ingredients such as G-CSF, GM-CSF, EPO, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13-cis-retinoic acid, or a pharmacologically active mutant or derivative thereof, or a combination thereof. Examples of the additional active ingredients include, but are not limited to, those disclosed herein (see, e.g., section 5.2).

Kits of the invention can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

## 6. EXAMPLES

The following studies are intended to further illustrate the invention without limiting its scope.

### 6.1. Clinical Studies in MDS Patients

#### Study 1

A Phase I trial was conducted in patients with MDS (IPSS score  $\geq 1.5$ , or FAB or WHO classification with  $\geq 5\%$  myeloblasts). Patients were treated using below dosing schedules.

Dose Level	Azacitidine Schedule	Lenalidomide Schedule
1	75 mg/m <sup>2</sup> SC days 1-5	5 mg PO days 1-14
2	75 mg/m <sup>2</sup> SC days 1-5	5 mg PO days 1-21
3	75 mg/m <sup>2</sup> SC days 1-5	10 mg PO days 1-21
4	50 mg/m <sup>2</sup> SC days 1-5, 8-12	5 mg PO days 1-14
5	50 mg/m <sup>2</sup> SC days 1-5, 8-12	5 mg PO days 1-21
6	50 mg/m <sup>2</sup> SC days 1-5, 8-12	10 mg PO days 1-21

Lenalidomide and Azacitidine were cyclically administered to patients with MDS. Cycling therapy involved the administration of the agents for a period of time, followed by rest for a period of time, and repeating this sequential administration. Cycles lasted 28 days, to a maximum of 7 cycles of therapy. The primary endpoint was to determine the maximum tolerated dose and dose-limiting toxicities of the combination. The secondary endpoint was response as defined by the Modified International Working Group (IWG). The combination of lenalidomide and Azacitidine was well-tolerated and the results showed efficacy in MDS patients.

#### Study 2

Patients receive treatment with lenalidomide at a oral dose of 5-25 mg daily on days 1 through 21, and Azacitidine at a

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dose of 25-75 mg/m<sup>2</sup>/d subcutaneously (SC) or intravenously (IV) on days 1 through 7 in cycles of 28 days. Patients receive treatments until disease progression or unacceptable toxicity. The combination of lenalidomide and Azacitidine is well tolerated with encouraging clinical activity. The results of this study indicate that the combination of lenalidomide and Azacitidine provides a highly effective treatment for MDS. These agents may complement each other by targeting both the bone marrow microenvironment and hypomethylating action on the malignant clone.

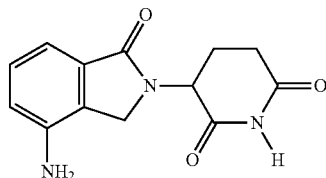
#### Study 3

Patients receive continuous treatment with lenalidomide at a oral dose of 5-25 mg daily on days 1 through 21, and Azacitidine at a oral dose of 120 mg per day on days 1 through 7 in every 28 days cycle. Patients receive treatments until disease progression or unacceptable toxicity. The results indicate that the combination dosing regimen of lenalidomide and oral Azacitidine is active and well tolerated, with a manageable side effect profile. This therapy provides a highly effective treatment for MDS.

Embodiments of the invention described herein are only a sampling of the scope of the invention. The full scope of the invention is better understood with reference to the attached claims.

#### What is claimed is:

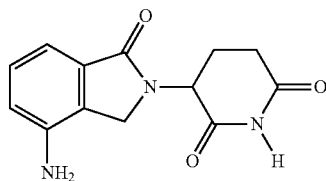
1. A method of treating myelodysplastic syndrome, which comprises administering to a patient in need thereof about 1 mg to about 25 mg per day of a compound having the formula:



or a pharmaceutically acceptable salt, solvate or stereoisomer thereof.

2. The method of claim 1, wherein the myelodysplastic syndrome is refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, or chronic myelomonocytic leukemia.

3. The method of claim 1, wherein the compound is



and is not a pharmaceutically acceptable salt, solvate or stereoisomer.

4. The method of claim 1, wherein the compound is a pharmaceutically acceptable salt.

5. The method of claim 1, wherein the compound is a pharmaceutically acceptable solvate.

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6. The method of claim 1, wherein the compound is a pharmaceutically acceptable stereoisomer.

7. The method of claim 1, wherein the patient is not previously treated for a myelodysplastic syndrome.

8. The method of claim 1, wherein the patient has been previously treated for a myelodysplastic syndrome.

9. The method of claim 1, which further comprises administering a therapeutically effective amount of a second active agent.

10. The method of claim 9, wherein the second active agent is dexamethasone.

11. The method of claim 9, wherein the second active agent is azacitidine.

12. The method of claim 1, wherein the compound is administered before, during or after transplanting umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow in the patient.

13. The method of claim 1, wherein the compound is administered cyclically.

14. The method of claim 1, wherein compound is administered for 21 consecutive days followed by seven consecutive days of rest in a 28 day cycle.

15. The method of claim 1, which comprises cyclically administering the compound until disease progression or unacceptable toxicity.

16. The method of claim 1, wherein the compound is administered in an amount of about 2.5 mg per day.

17. The method of claim 1, wherein the compound is administered in an amount of about 5 mg per day.

18. The method of claim 1, wherein the compound is administered in an amount of about 10 mg per day.

19. The method of claim 1, wherein the compound is administered in an amount of about 15 mg per day.

20. The method of claim 1, wherein the compound is administered in an amount of about 20 mg per day.

21. The method of claim 1, wherein the compound is administered in an amount of about 25 mg per day.

22. The method of claim 1, wherein the compound is administered in an amount from about 5 mg per day to about 25 mg per day for 21 consecutive days followed by seven consecutive days of rest in a 28 day cycle.

23. The method of claim 1, wherein the compound is administered in an amount of about 10 mg per day or about 15 mg per day for 21 consecutive days followed by seven consecutive days of rest in a 28 day cycle.

24. The method of claim 1, wherein the compound is administered orally.

25. The method of claim 24, wherein the compound is administered in the form of a capsule or tablet.

26. The method of claim 25, wherein the compound is administered in a capsule in an amount from about 1 mg to about 25 mg.

27. The method of claim 25, wherein the compound is administered in a capsule in an amount of about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg or about 25 mg.

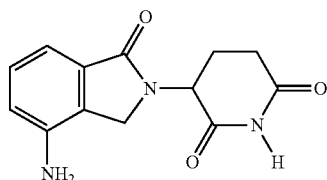
28. A method of treating transfusion dependent anemia due to low to intermediate-1-risk myelodysplastic syndrome, which comprises administering to a patient in need thereof



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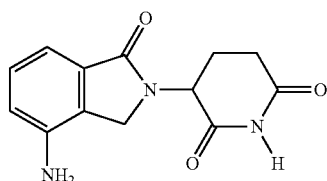
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about 1 mg to about 25 mg per day of a compound having the formula:



or a pharmaceutically acceptable salt, solvate or stereoisomer thereof.

**29.** The method of claim **28**, wherein the compound is



and is not a pharmaceutically acceptable salt, solvate or stereoisomer.

**30.** The method of claim **28**, wherein the compound is a pharmaceutically acceptable salt.

**31.** The method of claim **28**, wherein the compound is a pharmaceutically acceptable solvate.

**32.** The method of claim **28**, wherein the compound is a pharmaceutically acceptable stereoisomer.

**33.** The method of claim **28**, wherein the patient is not previously treated for a myelodysplastic syndrome.

**34.** The method of claim **28**, wherein the patient has been previously treated for a myelodysplastic syndrome.

**35.** The method of claim **28**, which further comprises administering a therapeutically effective amount of a second active agent.

**36.** The method of claim **35**, wherein the second active agent is dexamethasone.

**37.** The method of claim **35**, wherein the second active agent is azacitidine.

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**38.** The method of claim **28**, wherein the compound is administered before, during or after transplanting umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow in the patient.

**39.** The method of claim **28**, wherein the compound is administered cyclically.

**40.** The method of claim **28**, wherein compound is administered for 21 consecutive days followed by seven consecutive days of rest in a 28 day cycle.

**41.** The method of claim **28**, which comprises cyclically administering the compound until disease progression or unacceptable toxicity.

**42.** The method of claim **28**, wherein the compound is administered in an amount of about 2.5 mg per day.

**43.** The method of claim **28**, wherein the compound is administered in an amount of about 5 mg per day.

**44.** The method of claim **28**, wherein the compound is administered in an amount of about 10 mg per day.

**45.** The method of claim **28**, wherein the compound is administered in an amount of about 15 mg per day.

**46.** The method of claim **28**, wherein the compound is administered in an amount of about 20 mg per day.

**47.** The method of claim **28**, wherein the compound is administered in an amount of about 25 mg per day.

**48.** The method of claim **28**, wherein the compound is administered in an amount from about 5 mg per day to about 25 mg per day for 21 consecutive days followed by seven consecutive days of rest in a 28 day cycle.

**49.** The method of claim **28**, wherein the compound is administered in an amount of about 10 mg per day or about 15 mg per day for 21 consecutive days followed by seven consecutive days of rest in a 28 day cycle.

**50.** The method of claim **28**, wherein the compound is administered orally.

**51.** The method of claim **50**, wherein the compound is administered in the form of a capsule or tablet.

**52.** The method of claim **51**, wherein the compound is administered in a capsule in an amount from about 1 mg to about 25 mg.

**53.** The method of claim **51**, wherein the compound is administered in a capsule in an amount of about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg or about 25 mg.

\* \* \* \* \*

# EXHIBIT I





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(12) **United States Patent**  
**Zeldis**

(10) **Patent No.:** **US 9,101,621 B2**  
(45) **Date of Patent:** **\*Aug. 11, 2015**

(54) **METHODS FOR TREATING MULTIPLE MYELOMA WITH 3-(4-AMINO-1-OXO-1,3-DIHYDRO-ISOINDOL-2-YL)-PIPERIDINE-2,6-DIONE AFTER STEM CELL TRANSPLANTATION**

*A61K 31/573* (2013.01); *A61K 31/675* (2013.01); *A61K 31/704* (2013.01); *A61K 31/7048* (2013.01); *A61K 35/12* (2013.01); *A61K 39/0011* (2013.01); *A61K 39/3955* (2013.01); *A61K 45/06* (2013.01)

(71) Applicant: **Celgene Corporation**, Summit, NJ (US)

(58) **Field of Classification Search**

(72) Inventor: **Jerome B. Zeldis**, Princeton, NJ (US)

CPC ..... *A61K 31/4035*; *A61K 31/425*; *A61K 31/445*; *A61K 31/454*

(73) Assignee: **Celgene Corporation**, Summit, NJ (US)

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

See application file for complete search history.

This patent is subject to a terminal disclaimer.

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(65) **Prior Publication Data**

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(57) **ABSTRACT**

Methods of treating, preventing and/or managing cancer as well as and diseases and disorders associated with, or characterized by, undesired angiogenesis are disclosed. Specific methods encompass the administration of an immunomodulatory compound alone or in combination with a second active ingredient. The invention further relates to methods of reducing or avoiding adverse side effects associated with chemotherapy, radiation therapy, hormonal therapy, biological therapy or immunotherapy which comprise the administration of an immunomodulatory compound. Pharmaceutical compositions, single unit dosage forms, and kits suitable for use in methods of the invention are also disclosed.

**21 Claims, 1 Drawing Sheet**

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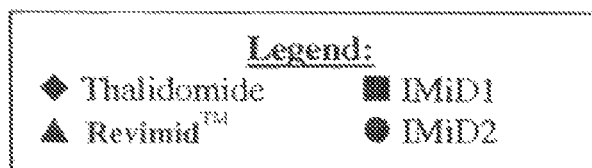
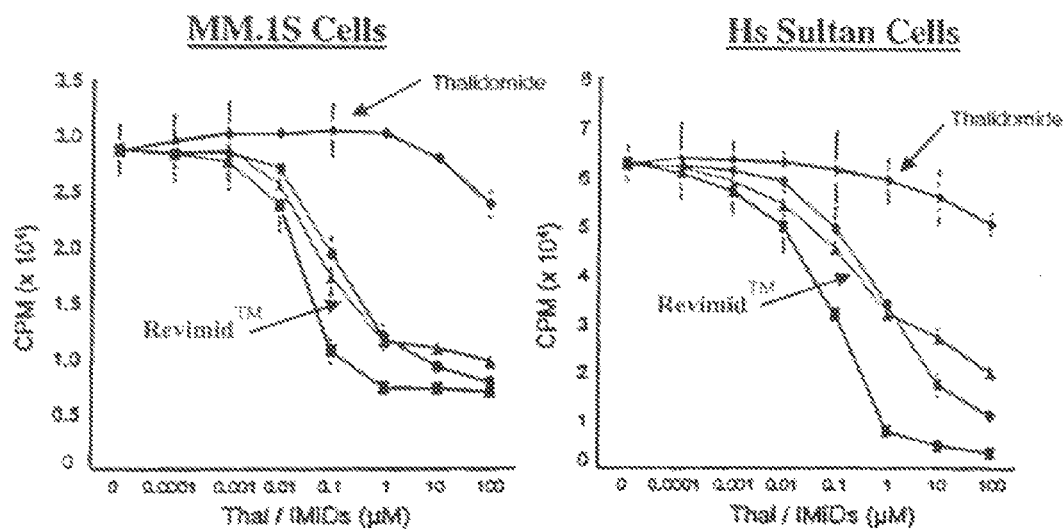
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**U.S. Patent****Aug. 11, 2015****US 9,101,621 B2****Effects of Revimid<sup>TM</sup> and Thalidomide on MM Cell Proliferation**



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1

**METHODS FOR TREATING MULTIPLE  
MYELOMA WITH 3-(4-AMINO-1-OXO-1,3-  
DIHYDRO-ISOINDOL-2-YL)-PIPERIDINE-  
2,6-DIONE AFTER STEM CELL  
TRANSPLANTATION**

This application is a continuation of U.S. patent application Ser. No. 14/201,069, filed Mar. 7, 2014, which is a continuation of U.S. patent application Ser. No. 13/782,728, filed Mar. 1, 2013, now U.S. Pat. No. 8,673,939, which is a continuation of U.S. patent application Ser. No. 13/488,888, filed Jun. 5, 2012, now U.S. Pat. No. 8,648,095, which is a continuation of U.S. patent application Ser. No. 12/640,702, filed Dec. 17, 2009, now U.S. Pat. No. 8,198,306, which is a continuation application of U.S. patent application Ser. No. 10/438,213, filed May 15, 2003, now U.S. Pat. No. 7,968,569, which claims the benefit of U.S. provisional application nos. 60/380,842, filed May 17, 2002, and 60/424,600, filed Nov. 6, 2002, the entireties of which are incorporated herein by reference.

## 1. FIELD OF THE INVENTION

This invention relates to methods of treating, preventing and/or managing specific cancers, and other diseases including, but not limited to, those associated with, or characterized by, undesired angiogenesis, by the administration of one or more immunomodulatory compounds alone or in combination with other therapeutics. In particular, the invention encompasses the use of specific combinations, or "cocktails," of drugs and other therapy, e.g., radiation to treat these specific cancers, including those refractory to conventional therapy. The invention also relates to pharmaceutical compositions and dosing regimens.

## 2. BACKGROUND OF THE INVENTION

### 2.1 Pathobiology of Cancer and Other Diseases

Cancer is characterized primarily by an increase in the number of abnormal cells derived from a given normal tissue, invasion of adjacent tissues by these abnormal cells, or lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites (metastasis). Clinical data and molecular biologic studies indicate that cancer is a multistep process that begins with minor preneoplastic changes, which may under certain conditions progress to neoplasia. The neoplastic lesion may evolve clonally and develop an increasing capacity for invasion, growth, metastasis, and heterogeneity, especially under conditions in which the neoplastic cells escape the host's immune surveillance. Roitt, I., Brostoff, J and Kale, D., *Immunology*, 17.1-17.12 (3rd ed., Mosby, St. Louis, Mo., 1993).

There is an enormous variety of cancers which are described in detail in the medical literature. Examples includes cancer of the lung, colon, rectum, prostate, breast, brain, and intestine. The incidence of cancer continues to climb as the general population ages, as new cancers develop, and as susceptible populations (e.g., people infected with AIDS or excessively exposed to sunlight) grow. A tremendous demand therefore exists for new methods and compositions that can be used to treat patients with cancer.

Many types of cancers are associated with new blood vessel formation, a process known as angiogenesis. Several of the mechanisms involved in tumor-induced angiogenesis have been elucidated. The most direct of these mechanisms is the secretion by the tumor cells of cytokines with angiogenic

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properties. Examples of these cytokines include acidic and basic fibroblastic growth factor (a,b-FGF), angiogenin, vascular endothelial growth factor (VEGF), and TNF- $\alpha$ . Alternatively, tumor cells can release angiogenic peptides through the production of proteases and the subsequent breakdown of the extracellular matrix where some cytokines are stored (e.g., b-FGF). Angiogenesis can also be induced indirectly through the recruitment of inflammatory cells (particularly macrophages) and their subsequent release of angiogenic cytokines (e.g., TNF- $\alpha$ , bFGF).

A variety of other diseases and disorders are also associated with, or characterized by, undesired angiogenesis. For example, enhanced or unregulated angiogenesis has been implicated in a number of diseases and medical conditions including, but not limited to, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, rubeosis (neovascularization of the angle), viral diseases, genetic diseases, inflammatory diseases, allergic diseases, and autoimmune diseases. Examples of such diseases and conditions include, but are not limited to: diabetic retinopathy; retinopathy of prematurity; corneal graft rejection; neovascular glaucoma; retrolental fibroplasia; and proliferative vitreoretinopathy.

Accordingly, compounds that can control angiogenesis or inhibit the production of certain cytokines, including TNF- $\alpha$ , may be useful in the treatment and prevention of various diseases and conditions.

### 2.2 Methods of Treating Cancer

Current cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient (see, for example, Stockdale, 1998, *Medicine*, vol. 3, Rubenstein and Federman, eds., Chapter 12, Section IV). Recently, cancer therapy could also involve biological therapy or immunotherapy. All of these approaches pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of a patient or may be unacceptable to the patient. Additionally, surgery may not completely remove neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue. Radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent. Although hormonal therapy can be effective, it is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of cancer cells. Biological therapies and immunotherapies are limited in number and may produce side effects such as rashes or swellings, flu-like symptoms, including fever, chills and fatigue, digestive tract problems or allergic reactions.

With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A majority of cancer chemotherapeutics act by inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division. Gilman et al., *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, Tenth Ed. (McGraw Hill, New York).

Despite availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks. Stockdale, *Medicine*, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. 10, 1998. Almost all chemotherapeutic agents are toxic, and chemotherapy causes significant, and often dangerous side effects including severe nausea, bone marrow depression, and immunosuppression. Additionally, even with administration of combinations of chemotherapeutic agents, many tumor

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cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even if those agents act by different mechanism from those of the drugs used in the specific treatment. This phenomenon is referred to as pleiotropic drug or multidrug resistance. Because of the drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

Other diseases or conditions associated with, or characterized by, undesired angiogenesis are also difficult to treat. However, some compounds such as protamine, heparin and steroids have been proposed to be useful in the treatment of certain specific diseases. Taylor et al., *Nature* 297:307 (1982); Folkman et al., *Science* 221:719 (1983); and U.S. Pat. Nos. 5,001,116 and 4,994,443. Thalidomide and certain derivatives of it have also been proposed for the treatment of such diseases and conditions. U.S. Pat. Nos. 5,593,990, 5,629,327, 5,712,291, 6,071,948 and 6,114,355 to D'Amato.

Still, there is a significant need for safe and effective methods of treating, preventing and managing cancer and other diseases and conditions, particularly for diseases that are refractory to standard treatments, such as surgery, radiation therapy, chemotherapy and hormonal therapy, while reducing or avoiding the toxicities and/or side effects associated with the conventional therapies.

### 2.3 IMIDS™

A number of studies have been conducted with the aim of providing compounds that can safely and effectively be used to treat diseases associated with abnormal production of TNF- $\alpha$ . See, e.g., Marriott, J. B., et al., *Expert Opin. Biol. Ther.* 1(4):1-8 (2001); G. W. Muller, et al., *Journal of Medicinal Chemistry* 39(17): 3238-3240 (1996); and G. W. Muller, et al., *Bioorganic & Medicinal Chemistry Letters* 8: 2669-2674 (1998). Some studies have focused on a group of compounds selected for their capacity to potentially inhibit TNF- $\alpha$  production by LPS stimulated PBMC. L. G. Corral, et al., *Ann. Rheum. Dis.* 58:(Suppl 1) 1107-1113 (1999). These compounds, which are referred to as IMiDs™ (Celgene Corporation) or Immunomodulatory Drugs, show not only potent inhibition of TNF- $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  and IL12 production. LPS induced IL6 is also inhibited by immunomodulatory compounds, albeit partially. These compounds are potent stimulators of LPS induced IL10. Id. Particular examples of IMiDs™ include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles described in U.S. Pat. Nos. 6,281,230 and 6,316,471, both to G. W. Muller, et al.

### 3. SUMMARY OF THE INVENTION

This invention encompasses methods of treating and preventing certain types of cancer, including primary and metastatic cancer, as well as cancers that are refractory or resistant to conventional chemotherapy. The methods comprise administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. The invention also encompasses methods of managing certain cancers (e.g., preventing or prolonging their recurrence, or lengthening the time of remission) which comprise administering to a patient in need of such management a prophylactically effective amount of an

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immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage cancer. Examples of such conventional therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention also encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are associated with, or characterized by, undesired angiogenesis, which comprise administering to a patient in need of such treatment, management or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In other methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage diseases or disorders associated with, or characterized by, undesired angiogenesis. Examples of such conventional therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention encompasses pharmaceutical compositions, single unit dosage forms, dosing regimens and kits which comprise an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second, or additional, active agent. Second active agents include specific combinations, or "cocktails," of drugs.

### 4. BRIEF DESCRIPTION OF FIGURE

FIG. 1 shows a comparison of the effects of 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide in inhibiting the proliferation of multiple myeloma (MM) cell lines in an in vitro study. The uptake of [<sup>3</sup>H]-thymidine by different MM cell lines (MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of the cell proliferation.

### 5. DETAILED DESCRIPTION OF THE INVENTION

A first embodiment of the invention encompasses methods of treating, managing, or preventing cancer which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with another drug ("second active agent") or method of treating, managing, or preventing cancer. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage cancer.

Another embodiment of the invention encompasses methods of treating, managing or preventing diseases and disorders



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ders other than cancer that are characterized by undesired angiogenesis. These methods comprise the administration of a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

Examples of diseases and disorders associated with, or characterized by, undesired angiogenesis include, but are not limited to, inflammatory diseases, autoimmune diseases, viral diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, and rubeosis (neovascularization of the angle).

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with a second active agent or method of treating, managing, or preventing the disease or condition. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage disease and conditions associated with, or characterized by, undesired angiogenesis.

The invention also encompasses pharmaceutical compositions (e.g., single unit dosage forms) that can be used in methods disclosed herein. Particular pharmaceutical compositions comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second active agent.

### 5.1 Immunomodulatory Compounds

Compounds used in the invention include immunomodulatory compounds that are racemic, stereomerically enriched or stereomerically pure, and pharmaceutically acceptable salts, solvates, hydrates, stereoisomers, clathrates, and prodrugs thereof. Preferred compounds used in the invention are small organic molecules having a molecular weight less than about 1,000 g/mol, and are not proteins, peptides, oligonucleotides, oligosaccharides or other macromolecules.

As used herein and unless otherwise indicated, the terms "immunomodulatory compounds" and "IMiDs™" (Celgene Corporation) encompasses small organic molecules that markedly inhibit TNF- $\alpha$ , LPS induced monocyte IL1 $\beta$  and IL12, and partially inhibit IL6 production. Specific immunomodulatory compounds are discussed below.

TNF- $\alpha$  is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation. TNF- $\alpha$  is responsible for a diverse range of signaling events within cells. TNF- $\alpha$  may play a pathological role in cancer. Without being limited by theory, one of the biological effects exerted by the immunomodulatory compounds of the invention is the reduction of synthesis of TNF- $\alpha$ . Immunomodulatory compounds of the invention enhance the degradation of TNF- $\alpha$  mRNA.

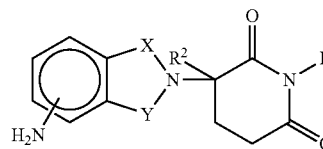
Further, without being limited by theory, immunomodulatory compounds used in the invention may also be potent co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. Immunomodulatory compounds of the invention may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the

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CD4+ T cell subset. In addition, the compounds preferably have anti-inflammatory properties, and efficiently co-stimulate T cells.

Specific examples of immunomodulatory compounds of the invention, include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl) isoindolines such as those described in U.S. Pat. No. 5,874,448; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolines described in U.S. Pat. No. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines (e.g., 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517; and a class of non-polypeptide cyclic amides disclosed in U.S. Pat. Nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S. Pat. Nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles such as those described in U.S. Pat. Nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those described in U.S. patent application Ser. No. 09/972,487 filed on Oct. 5, 2001, U.S. patent application Ser. No. 10/032,286 filed on Dec. 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents and patent applications identified herein are incorporated herein by reference. Immunomodulatory compounds of the invention do not include thalidomide.

Other specific immunomodulatory compounds of the invention include, but are not limited to, 1-oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein by reference. These compounds have the structure I:



in which one of X and Y is C=O, the other of X and Y is C=O or CH<sub>2</sub>, and R<sup>2</sup> is hydrogen or lower alkyl, in particular methyl. Specific immunomodulatory compounds include, but are not limited to:

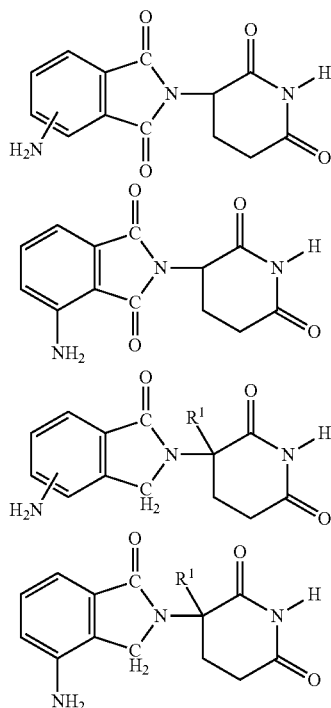
1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;  
1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;  
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;  
and  
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

Other specific immunomodulatory compounds of the invention belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles, such as those described in U.S. Pat. Nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (In-

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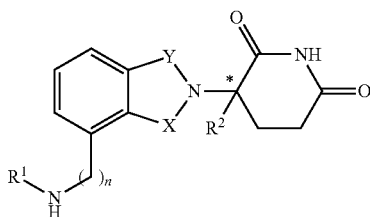
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ternational Publication No. WO 98/03502), each of which is incorporated herein by reference. Compounds representative of this class are of the formulas:



wherein R<sup>1</sup> is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (e.g. optically pure (R) or (S) enantiomers) of these compounds.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. Nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are incorporated herein by reference. Representative compounds are of formula II:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

R<sup>1</sup> is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, C(O)R<sup>3</sup>, C(S)R<sup>3</sup>, C(O)OR<sup>4</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, C(O)NHR<sup>3</sup>, C(S)NHR<sup>3</sup>, C(O)NR<sup>3</sup>R<sup>3</sup>, C(S)NR<sup>3</sup>R<sup>3</sup> or (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>;

R<sup>2</sup> is H, F, benzyl, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, or (C<sub>2</sub>-C<sub>8</sub>)alkynyl;

R<sup>3</sup> and R<sup>3'</sup> are independently (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-

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C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>0</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>, or C(O)OR<sup>5</sup>;

R<sup>4</sup> is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, (C<sub>1</sub>-C<sub>4</sub>)alkyl-OR<sup>5</sup>, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, or (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl;

R<sup>5</sup> is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, or (C<sub>2</sub>-C<sub>5</sub>)heteroaryl;

each occurrence of R<sup>6</sup> is independently H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>2</sub>-C<sub>5</sub>)heteroaryl, or (C<sub>0</sub>-C<sub>8</sub>)alkyl-C(O)O—R<sup>5</sup> or the R<sup>6</sup> groups can join to form a heterocycloalkyl group;

n is 0 or 1; and

\* represents a chiral-carbon center.

In specific compounds of formula II, when n is 0 then R<sup>1</sup> is (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, C(O)R<sup>3</sup>, C(O)OR<sup>4</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, C(S)NHR<sup>3</sup>, or (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>;

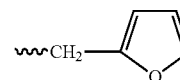
R<sup>2</sup> is H or (C<sub>1</sub>-C<sub>8</sub>)alkyl; and

R<sup>3</sup> is (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (C<sub>2</sub>-C<sub>8</sub>)alkenyl, (C<sub>2</sub>-C<sub>8</sub>)alkynyl, benzyl, aryl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>1</sub>-C<sub>6</sub>)heterocycloalkyl, (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>5</sub>-C<sub>8</sub>)alkyl-N(R<sup>6</sup>)<sub>2</sub>; (C<sub>0</sub>-C<sub>8</sub>)alkyl-NH—C(O)O—R<sup>5</sup>; (C<sub>1</sub>-C<sub>8</sub>)alkyl-OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-C(O)OR<sup>5</sup>, (C<sub>1</sub>-C<sub>8</sub>)alkyl-O(CO)R<sup>5</sup>, or C(O)OR<sup>5</sup>; and the other variables have the same definitions.

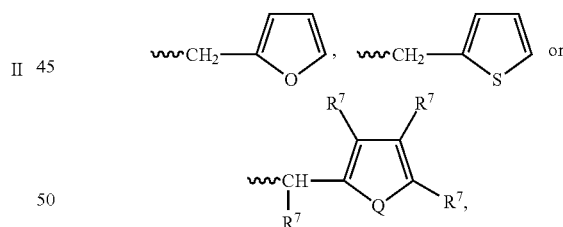
In other specific compounds of formula II, R<sup>2</sup> is H or (C<sub>1</sub>-C<sub>4</sub>)alkyl.

In other specific compounds of formula II, R<sup>1</sup> is (C<sub>1</sub>-C<sub>8</sub>)alkyl or benzyl.

In other specific compounds of formula II, R<sup>1</sup> is H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, benzyl, CH<sub>2</sub>OCH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, or



In another embodiment of the compounds of formula II, R<sup>1</sup> is



wherein Q is O or S, and each occurrence of R<sup>7</sup> is independently H, (C<sub>1</sub>-C<sub>8</sub>)alkyl, benzyl, CH<sub>2</sub>OCH<sub>3</sub>, or CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>.

In other specific compounds of formula II, R<sup>1</sup> is C(O)R<sup>3</sup>.

In other specific compounds of formula II, R<sup>3</sup> is (C<sub>0</sub>-C<sub>4</sub>)alkyl-(C<sub>2</sub>-C<sub>5</sub>)heteroaryl, (C<sub>1</sub>-C<sub>8</sub>)alkyl, aryl, or (C<sub>0</sub>-C<sub>4</sub>)alkyl-OR<sup>5</sup>.

In other specific compounds of formula II, heteroaryl is pyridyl, furyl, or thienyl.

In other specific compounds of formula II, R<sup>1</sup> is C(O)OR<sup>4</sup>.

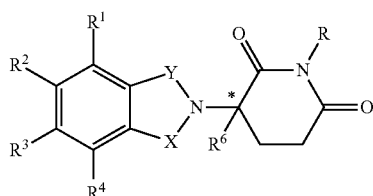
In other specific compounds of formula II, the H of C(O)NHC(O) can be replaced with (C<sub>1</sub>-C<sub>4</sub>)alkyl, aryl, or benzyl.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. No. 09/781,179, International Publication No. WO 98/54170, and U.S. Pat. No. 6,395,754,

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each of which are incorporated herein by reference. Representative compounds are of formula III:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

R is H or CH<sub>2</sub>OCOR';

(i) each of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup>, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> is nitro or —NHR<sup>5</sup> and the remaining of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> are hydrogen;

R<sup>5</sup> is hydrogen or alkyl of 1 to 8 carbons

R<sup>6</sup> hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro;

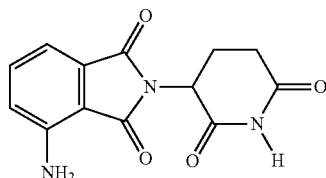
R' is R<sup>7</sup>—CHR<sup>10</sup>—N(R<sup>8</sup>R<sup>9</sup>);

R<sup>7</sup> is m-phenylene or p-phenylene or —(C<sub>n</sub>H<sub>2n</sub>)— in which n has a value of 0 to 4;

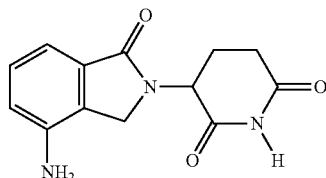
each of R<sup>8</sup> and R<sup>9</sup> taken independently of the other is hydrogen or alkyl of 1 to 8 carbon atoms, or R<sup>8</sup> and R<sup>9</sup> taken together are tetramethylene, pentamethylene, hexamethylene, or —CH<sub>2</sub>CH<sub>2</sub>[X]X<sub>1</sub>CH<sub>2</sub>CH<sub>2</sub>— in which [X]X<sub>1</sub> is —O—, —S—, or —NH—;

R<sup>10</sup> is hydrogen, alkyl of 1 to 8 carbon atoms, or phenyl; and \* represents a chiral-carbon center.

The most preferred immunomodulatory compounds of the invention are 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione. The compounds can be obtained via standard, synthetic methods (see e.g., U.S. Pat. No. 5,635, 517, incorporated herein by reference). The compounds are available from Celgene Corporation, Warren, N.J. 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isindoline-1,3-dione (ACTIMID™) has the following chemical structure:



The compound 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione (REVIMID™) has the following chemical structure:



Compounds of the invention can either be commercially purchased or prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized

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or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry techniques.

As used herein and unless otherwise indicated, the term “pharmaceutically acceptable salt” encompasses non-toxic acid and base addition salts of the compound to which the term refers. Acceptable non-toxic acid addition salts include those derived from organic and inorganic acids or bases known in the art, which include, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embolic acid, enanthic acid, and the like.

Compounds that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds are those that form non-toxic base addition salts, i.e., salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), lysine, and procaine.

As used herein and unless otherwise indicated, the term “prodrug” means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of immunomodulatory compounds of the invention that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of immunomodulatory compounds of the invention that comprise —NO, —NO<sub>2</sub>, —ONO, or —ONO<sub>2</sub> moieties. Prodrugs can typically be prepared using well-known methods, such as those described in 1 *Burger's Medicinal Chemistry and Drug Discovery*, 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995), and *Design of Prodrugs* (H. Bundgaard ed., Elsevier, New York 1985).

As used herein and unless otherwise indicated, the terms “biohydrolyzable amide,” “biohydrolyzable ester,” “biohydrolyzable carbamate,” “biohydrolyzable carbonate,” “biohydrolyzable ureide,” “biohydrolyzable phosphate” mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxymethyl, acetoxylethyl, aminocarbonyloxymethyl, pivaloyloxymethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyloxyethyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates

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include, but are not limited to, lower alkylamines, substituted ethylenediamines, amino acids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

Various immunomodulatory compounds of the invention contain one or more chiral centers, and can exist as racemic mixtures of enantiomers or mixtures of diastereomers. This invention encompasses the use of stereomerically pure forms of such compounds, as well as the use of mixtures of those forms. For example, mixtures comprising equal or unequal amounts of the enantiomers of a particular immunomodulatory compounds of the invention may be used in methods and compositions of the invention. These isomers may be asymmetrically synthesized or resolved using standard techniques such as chiral columns or chiral resolving agents. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. As used herein and unless otherwise indicated, the term "stereomerically enriched" means a composition that comprises greater than about 60% by weight of one stereoisomer of a compound, preferably greater than about 70% by weight, more preferably greater than about 80% by weight of one stereoisomer of a compound. As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center. Similarly, the term "stereomerically enriched" means a stereomerically enriched composition of a compound having one chiral center.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

## 5.2 Second Active Agents

Immunomodulatory compounds can be combined with other pharmacologically active compounds ("second active agents") in methods and compositions of the invention. It is believed that certain combinations work synergistically in the

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treatment of particular types of cancer and certain diseases and conditions associated with, or characterized by, undesired angiogenesis. Immunomodulatory compounds can also work to alleviate adverse effects associated with certain second active agents, and some second active agents can be used to alleviate adverse effects associated with immunomodulatory compounds.

One or more second active ingredients or agents can be used in the methods and compositions of the invention together with an immunomodulatory compound. Second active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules).

Examples of large molecule active agents include, but are not limited to, hematopoietic growth factors, cytokines, and monoclonal and polyclonal antibodies. Typical large molecule active agents are biological molecules, such as naturally occurring or artificially made proteins. Proteins that are particularly useful in this invention include proteins that stimulate the survival and/or proliferation of hematopoietic precursor cells and immunologically active poietic cells in vitro or in vivo. Others stimulate the division and differentiation of committed erythroid progenitors in cells in vitro or in vivo. Particular proteins include, but are not limited to: interleukins, such as IL-2 (including recombinant IL-2 ("rIL2")) and canarypox TL-2), IL-10, IL-12, and IL-18; interferons, such as interferon alfa-2a, interferon alfa-2b, interferon alfa-n1, interferon alfa-n3, interferon beta-1 a, and interferon gamma-I b; GM-CSF and GM-CSF; and EPO.

Particular proteins that can be used in the methods and compositions of the invention include, but are not limited to: filgrastim, which is sold in the United States under the trade name Neupogen® (Amgen, Thousand Oaks, Calif.); sargramostim, which is sold in the United States under the trade name Leukine® (Immunex, Seattle, Wash.); and recombinant EPO, which is sold in the United States under the trade name Epogen® (Amgen, Thousand Oaks, Calif.).

Recombinant and mutated forms of GM-CSF can be prepared as described in U.S. Pat. Nos. 5,391,485; 5,393,870; and 5,229,496; all of which are incorporated herein by reference. Recombinant and mutated forms of G-CSF can be prepared as described in U.S. Pat. Nos. 4,810,643; 4,999,291; 5,528,823; and 5,580,755; all of which are incorporated herein by reference.

This invention encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term "mutants" are proteins that lack carbohydrate moieties normally present in their naturally occurring forms (e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001).

Antibodies that can be used in combination with compounds of the invention include monoclonal and polyclonal antibodies. Examples of antibodies include, but are not limited to, trastuzumab (Herceptin®), rituximab (Rituxan®), bevacizumab (Avastin™), pertuzumab (Omnitarg™), tositumomab (Bexxar®), edrecolomab (Panorex®), and G250.



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Compounds of the invention can also be combined with, or used in combination with, anti-TNF- $\alpha$  antibodies.

Large molecule active agents may be administered in the form of anti-cancer vaccines. For example, vaccines that secrete, or cause the secretion of, cytokines such as IL-2, G-CSF, and GM-CSF can be used in the methods, pharmaceutical compositions, and kits of the invention. See, e.g., Emens, L. A., et al., *Curr. Opin. Mol. Ther.* 3(1):77-84 (2001).

In one embodiment of the invention, the large molecule active agent reduces, eliminates, or prevents an adverse effect associated with the administration of an immunomodulatory compound. Depending on the particular immunomodulatory compound and the disease or disorder being treated, adverse effects can include, but are not limited to, drowsiness and somnolence, dizziness and orthostatic hypotension, neutropenia, infections that result from neutropenia, increased HIV-viral load, bradycardia, Stevens-Johnson Syndrome and toxic epidermal necrolysis, and seizures (e.g., grand mal convulsions). A specific adverse effect is neutropenia.

Second active agents that are small molecules can also be used to alleviate adverse effects associated with the administration of an immunomodulatory compound. However, like some large molecules, many are believed to be capable of providing a synergistic effect when administered with (e.g., before, after or simultaneously) an immunomodulatory compound. Examples of small molecule second active agents include, but are not limited to, anti-cancer agents, antibiotics, immunosuppressive agents, and steroids.

Examples of anti-cancer agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; amet-antrone acetate; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; celecoxib (COX-2 inhibitor); chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitucin; enloplatin; enpromate; epiropidine; epirubicin hydrochloride; erbulazole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; iroplatin; irinotecan; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarazole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprostol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedpa; mitidomide; mitocarcin; mitocromin; mitogillin; mitomycin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; pipsulfan; piroxantrone hydrochloride;

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plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiro-mustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; taxotere; tegafur; telox-antrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; tricitribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vingly-cinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; and zorubicin hydrochloride.

Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abirater-one; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angio-genesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligo-nucleotides; aphidicolin glycinate; apoptosis gene modula-tors; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azas-ctron; azatoxin; azatyrosine; baccatin ITT derivatives; bal-anol; batimastat; BCR/ABL antagonists; benzochlorins; ben-zoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicaluta-mide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin deriva-tives; capecitabine; carboxamide-amino-triazole; carboxya-midotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroqui-noxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismy-cin B; combretastatin A4; combretastatin analogue; conage-nin; crambescidin 816; crisnatol; cryptophycin 8; cryptophy-cin A derivatives; curacin A; cyclopentantraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic fac-tor; cytotatin; dacliximab; decitabine; dehydrididemnin B; deslorelin; dexamethasone; dexifosfamide; dextrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnor-spermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamy-cin; diphenyl spiromustine; docetaxel; docosanol; dolas-etron; doxifluridine; doxorubicin; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolo-mab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antago-nists; etanidazole; etoposide phosphate; exemestane; fadroz-ole; fazarabine; fenretinide; filgrastim; flnasteride; fla-vopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcit-abine; glutathione inhibitors; hepsulfam; heregulin; hexam-ethylene bisacetamide; hypericin; ibandronic acid; idarubi-cin; idoxifene; idramantone; ilmofofosine; ilomastat; imatinib (e.g., Gleevec®), imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon

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agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mitogazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; Erbitux, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullin; oblimersen (Genasense®); O<sup>6</sup>-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfimericin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; translation inhibitors; tretinoin; triacetylluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine

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kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Specific second active agents include, but are not limited to, oblimersen (Genasense®), remicade, docetaxel, celecoxib, melphalan, dexamethasone (Decadron®), steroids, gemcitabine, cisplatin, temozolomide, etoposide, cyclophosphamide, temodar, carboplatin, procarbazine, gliadel, tamoxifen, topotecan, methotrexate, Arisa®, taxol, taxotere, fluorouracil, leucovorin, irinotecan, xeloda, CPT-11, interferon alpha, pegylated interferon alpha (e.g., PEG INTRON-A), capecitabine, cisplatin, thiotepa, fludarabine, carboplatin, liposomal daunorubicin, cytarabine, doxetaxol, paclitaxel, vinblastine, IL-2, GM-CSF, dacarbazine, vinorelbine, zoledronic acid, palmitronate, biaxin, busulphan, prednisone, bisphosphonate, arsenic trioxide, vincristine, doxorubicin (Doxil®), paclitaxel, ganciclovir, adriamycin, estramustine sodium phosphate (Emcyt®), sulindac, and etoposide.

### 5.3 Methods of Treatments and Prevention

Methods of this invention encompass methods of treating, preventing and/or managing various types of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis. As used herein, unless otherwise specified, the term “treating” refers to the administration of a compound of the invention or other additional active agent after the onset of symptoms of the particular disease or disorder. As used herein, unless otherwise specified, the term “preventing” refers to the administration prior to the onset of symptoms, particularly to patients at risk of cancer, and other diseases and disorders associated with, or characterized by, undesired angiogenesis. The term “prevention” includes the inhibition of a symptom of the particular disease or disorder. Patients with familial history of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis are preferred candidates for preventive regimens. As used herein and unless otherwise indicated, the term “managing” encompasses preventing the recurrence of the particular disease or disorder in a patient who had suffered from it, and/or lengthening the time a patient who had suffered from the disease or disorder remains in remission.

As used herein, the term “cancer” includes, but is not limited to, solid tumors and blood born tumors. The term “cancer” refers to disease of skin tissues, organs, blood, and vessels, including, but not limited to, cancers of the bladder, bone or blood, brain, breast, cervix, chest, colon, endometrium, esophagus, eye, head, kidney, liver, lymph nodes, lung, mouth, neck, ovaries, pancreas, prostate, rectum, stomach, testis, throat, and uterus. Specific cancers include, but are not limited to, advanced malignancy, amyloidosis, neuroblastoma, meningioma, hemangiopericytoma, multiple brain metastase, glioblastoma multiforms, glioblastoma, brain stem glioma, poor prognosis malignant brain tumor, malignant glioma, recurrent malignant glioma, anaplastic astrocytoma, anaplastic oligodendroglioma, neuroendocrine tumor, rectal adenocarcinoma, Dukes C & D colorectal cancer, unresectable colorectal carcinoma, metastatic hepatocellular carcinoma, Kaposi’s sarcoma, karotype acute myeloblastic leukemia, Hodgkin’s lymphoma, non-Hodgkin’s lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma, low grade follicular lymphoma, malignant melanoma, malignant mesothelioma, malignant pleural effusion mesothelioma syndrome,



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peritoneal carcinoma, papillary serous carcinoma, gynecologic sarcoma, soft tissue sarcoma, scleroderma, cutaneous vasculitis, Langerhans cell histiocytosis, leiomyosarcoma, fibrodysplasia ossificans progressive, hormone refractory prostate cancer, resected high-risk soft tissue sarcoma, unresectable hepatocellular carcinoma, Waldenstrom's macroglobulinemia, smoldering myeloma, indolent myeloma, fallopian tube cancer, androgen independent prostate cancer, androgen dependent stage IV non-metastatic prostate cancer, hormone-insensitive prostate cancer, chemotherapy-insensitive prostate cancer, papillary thyroid carcinoma, follicular thyroid carcinoma, medullary thyroid carcinoma, and leiomyoma. In a specific embodiment, the cancer is metastatic. In another embodiment, the cancer is refractory or resistance to chemotherapy or radiation; in particular, refractory to thalidomide.

As used herein to refer to diseases and conditions other than cancer, the terms "diseases or disorders associated with, or characterized by, undesired angiogenesis," "diseases or disorders associated with undesired angiogenesis," and "diseases or disorders characterized by undesired angiogenesis" refer to diseases, disorders and conditions that are caused, mediated or attended by undesired, unwanted or uncontrolled angiogenesis, including, but not limited to, inflammatory diseases, autoimmune diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, chorioidal neovascular diseases, and retina neovascular diseases.

Examples of such diseases or disorders associated with undesired angiogenesis include, but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, proliferative vitreoretinopathy, trachoma, myopia, optic pits, epidemic keratoconjunctivitis, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, syphilis, lipid degeneration, bacterial ulcer, fungal ulcer, Herpes simplex infection, Herpes zoster infection, protozoan infection, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marigial keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, peripheral radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Behcet's disease, retinitis, choroiditis, presumed ocular histoplasmosis, Bests disease, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, rubeosis, sarcoidosis, sclerosis, soriatis, psoriasis, primary sclerosing cholangitis, proctitis, primary biliary srosis, idiopathic pulmonary fibrosis, and alcoholic hepatitis.

In specific embodiments of the invention, diseases or disorders associated with undesired angiogenesis do not include congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, myocardial infarction, HIV, hepatitis, adult respiratory distress syndrome, bone-resorption disease, chronic obstructive pulmonary diseases, chronic pulmonary inflammatory disease, dermatitis, cystic fibrosis, septic shock, sepsis, endotoxic shock, hemodynamic shock, sepsis syndrome, post ischemic reperfusion injury, meningitis, psoriasis, fibrotic disease, cachexia, graft rejection, rheumatoid spondylitis, osteoporosis, Crohn's disease, ulcerative colitis, inflammatory-bowel disease, multiple sclerosis, systemic lupus erythrematosus, erythema nodosum leprosum in leprosy, radiation damage, asthma, hyperoxic alveolar injury, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

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This invention encompasses methods of treating patients who have been previously treated for cancer or diseases or disorders associated with, or characterized by, undesired angiogenesis, but are non-responsive to standard therapies, as well as those who have not previously been treated. The invention also encompasses methods of treating patients regardless of patient's age, although some diseases or disorders are more common in certain age groups. The invention further encompasses methods of treating patients who have undergone surgery in an attempt to treat the disease or condition at issue, as well as those who have not. Because patients with cancer and diseases and disorders characterized by undesired angiogenesis have heterogenous clinical manifestations and varying clinical outcomes, the treatment given to a patient may vary, depending on his/her prognosis. The skilled clinician will be able to readily determine without undue experimentation specific secondary agents, types of surgery, and types of non-drug based standard therapy that can be effectively used to treat an individual patient with cancer and other diseases or disorders.

Methods encompassed by this invention comprise administering one or more immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, to a patient (e.g., a human) suffering, or likely to suffer, from cancer or a disease or disorder mediated by undesired angiogenesis.

In one embodiment of the invention, an immunomodulatory compound of the invention can be administered orally and in single or divided daily doses in an amount of from about 0.10 to about 150 mg/day. In a particular embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of from about 0.1 to about 1 mg per day, or alternatively from about 0.1 to about 5 mg every other day. In a preferred embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered in an amount of from about 5 to 25 mg per day, or alternatively from about 10 to about 50 mg every other day.

In a specific embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of about 1, 2, or 5 mg per day to patients with relapsed multiple myeloma. In a particular embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered initially in an amount of 5 mg/day and the dose can be escalated every week to 10, 20, 25, 30 and 50 mg/day. In a specific embodiment, Revimid™ can be administered in an amount of up to about 30 mg/day to patients with solid tumor. In a particular embodiment, Revimid™ can be administered in an amount of up to about 40 mg/day to patients with glioma.

### 5.3.1 Combination Therapy with a Second Active Agent

Specific methods of the invention comprise administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with one or more second active agents, and/or in combination with radiation therapy, blood transfusions, or surgery. Examples of immunomodulatory compounds of the invention are disclosed herein (see, e.g., section 5.1). Examples of second active agents are also disclosed herein (see, e.g., section 5.2).

Administration of the immunomodulatory compounds and the second active agents to a patient can occur simultaneously or sequentially by the same or different routes of administra-

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tion. The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. A preferred route of administration for an immunomodulatory compound of the invention is orally. Preferred routes of administration for the second active agents or ingredients of the invention are known to those of ordinary skill in the art. See, e.g., *Physicians' Desk Reference*, 1755-1760 (56<sup>th</sup> ed., 2002).

In one embodiment of the invention, the second active agent is administered intravenously or subcutaneously and once or twice daily in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. The specific amount of the second active agent will depend on the specific agent used, the type of disease being treated or managed, the severity and stage of disease, and the amount(s) of immunomodulatory compounds of the invention and any optional additional active agents concurrently administered to the patient. In a particular embodiment, the second active agent is oblimersen (Genasense®), GM-CSF, G-CSF, EPO, taxotere, irinotecan, dacarbazine, transretinoic acid, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, vincristine, doxorubicin, COX-2 inhibitor, IL2, IL8, IL18, IFN, Ara-C, vinorelbine, or a combination thereof.

In a particular embodiment, GM-CSF, G-CSF or EPO is administered subcutaneously during about five days in a four or six week cycle in an amount of from about 1 to about 750 mg/m<sup>2</sup>/day, preferably in an amount of from about 25 to about 500 mg/m<sup>2</sup>/day, more preferably in an amount of from about 50 to about 250 mg/m<sup>2</sup>/day, and most preferably in an amount of from about 50 to about 200 mg/m<sup>2</sup>/day. In a certain embodiment, GM-CSF may be administered in an amount of from about 60 to about 500 mcg/m<sup>2</sup> intravenously over 2 hours, or from about 5 to about 12 mcg/m<sup>2</sup>/day subcutaneously. In a specific embodiment, G-CSF may be administered subcutaneously in an amount of about 1 mcg/kg/day initially and can be adjusted depending on rise of total granulocyte counts. The maintenance dose of G-CSF may be administered in an amount of about 300 (in smaller patients) or 480 mcg subcutaneously. In a certain embodiment, EPO may be administered subcutaneously in an amount of 10,000 Unit 3 times per week.

In another embodiment, Revimid™ in an amount of about 25 mg/d and dacarbazine in an amount of about from 200 to 1,000 mg/m<sup>2</sup>/d are administered to patients with metastatic malignant melanoma. In a specific embodiment, Revimid™ is administered in an amount of from about 5 to about 25 mg/d to patients with metastatic malignant melanoma whose disease has progressed on treatment with dacarbazine, IL-2 or IFN. In a specific embodiment, Revimid™ is administered to patients with relapsed or refractory multiple myeloma in an amount of about 15 mg/d twice a day or about 30 mg/d four times a day in a combination with dexamethasone.

In another embodiment, an immunomodulatory compound is administered with melphalan and dexamethasone to patients with amyloidosis. In a specific embodiment, an immunomodulatory compound of the invention and steroids can be administered to patients with amyloidosis.

In another embodiment, an immunomodulatory compound is administered with gemcitabine and cisplatin to patients with locally advanced or metastatic transitional cell bladder cancer.

In another embodiment, an immunomodulatory compound is administered in combination with a second active ingredient as follows: temozolomide to pediatric patients with

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relapsed or progressive brain tumors or recurrent neuroblastoma; celecoxib, etoposide and cyclophosphamide for relapsed or progressive CNS cancer; temodar to patients with recurrent or progressive meningioma, malignant meningioma, hemangiopericytoma, multiple brain metastases, relapsed brain tumors, or newly diagnosed glioblastoma multiforms; irinotecan to patients with recurrent glioblastoma; carboplatin to pediatric patients with brain stem glioma; procarbazine to pediatric patients with progressive malignant gliomas; cyclophosphamide to patients with poor prognosis malignant brain tumors, newly diagnosed or recurrent glioblastoma multiforms; Gliadel® for high grade recurrent malignant gliomas; temozolomide and tamoxifen for anaplastic astrocytoma; or topotecan for gliomas, glioblastoma, anaplastic astrocytoma or anaplastic oligodendroglioma.

In another embodiment, an immunomodulatory compound is administered with methotrexate and cyclophosphamide to patients with metastatic breast cancer.

In another embodiment, an immunomodulatory compound is administered with temozolomide to patients with neuroendocrine tumors.

In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with recurrent or metastatic head or neck cancer. In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with pancreatic cancer.

In another embodiment, an immunomodulatory compound is administered to patients with colon cancer in combination with Arisa®, taxol and/or taxotere.

In another embodiment, an immunomodulatory compound is administered with capecitabine to patients with refractory colorectal cancer or patients who fail first line therapy or have poor performance in colon or rectal adenocarcinoma.

In another embodiment, an immunomodulatory compound is administered in combination with fluorouracil, leucovorin, and irinotecan to patients with Dukes C & D colorectal cancer or to patients who have been previously treated for metastatic colorectal cancer.

In another embodiment, an immunomodulatory compound is administered to patients with refractory colorectal cancer in combination with capecitabine, xeloda, and/or CPT-11.

In another embodiment, an immunomodulatory compound of the invention is administered with capecitabine and irinotecan to patients with refractory colorectal cancer or to patients with unresectable or metastatic colorectal carcinoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with interferon alpha or capecitabine to patients with unresectable or metastatic hepatocellular carcinoma; or with cisplatin and thiotepa to patients with primary or metastatic liver cancer.

In another embodiment, an immunomodulatory compound is administered in combination with pegylated interferon alpha to patients with Kaposi's sarcoma.

In another embodiment, an immunomodulatory compound is administered in combination with fludarabine, carboplatin, and/or topotecan to patients with refractory or relapsed or high-risk acute myelogenous leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with liposomal daunorubicin, topotecan and/or cytarabine to patients with unfavorable karyotype acute myeloblastic leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with gemcitabine and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered in combination with carboplatin and irinotecan to

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patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered with doxetaxol to patients with non-small cell lung cancer who have been previously treated with carbo/VP 16 and radiotherapy.

In another embodiment, an immunomodulatory compound is administered in combination with carboplatin and/or taxotere, or in combination with carboplatin, paclitaxel and/or thoracic radiotherapy to patients with non-small cell lung cancer. In a specific embodiment, an immunomodulatory compound is administered in combination with taxotere to patients with stage IIIB or TV non-small cell lung cancer.

In another embodiment, an immunomodulatory compound of the invention is administered in combination with oblimersen (Genasense®) to patients with small cell lung cancer.

In another embodiment, an immunomodulatory compound is administered alone or in combination with a second active ingredient such as vinblastine or fludarabine to patients with various types of lymphoma, including, but not limited to, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma or relapsed or refractory low grade follicular lymphoma.

In another embodiment, an immunomodulatory compound is administered in combination with taxotere, IL-2, IFN, GM-CSF, and/or dacarbazine to patients with various types or stages of melanoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with vinorelbine to patients with malignant mesothelioma, or stage IIIB non-small cell lung cancer with pleural implants or malignant pleural effusion mesothelioma syndrome.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of multiple myeloma in combination with dexamethasone, zoledronic acid, palmitronate, GM-CSF, biacin, vinblastine, melphalan, busulphan, cyclophosphamide, IFN, palmitronate, prednisone, bisphosphonate, celecoxib, arsenic trioxide, PEG INTRON-A, vincristine, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with relapsed or refractory multiple myeloma in combination with doxorubicin (Doxil®), vincristine and/or dexamethasone (Decadron®).

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of ovarian cancer such as peritoneal carcinoma, papillary serous carcinoma, refractory ovarian cancer or recurrent ovarian cancer, in combination with taxol, carboplatin, doxorubicin, gemcitabine, cisplatin, xeloda, paclitaxel, dexamethasone, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of prostate cancer, in combination with xeloda, 5 FU/LV, gemcitabine, irinotecan plus gemcitabine, cyclophosphamide, vincristine, dexamethasone, GM-CSF, celecoxib, taxotere, ganciclovir, paclitaxel, adriamycin, docetaxel, estramustine, Emcyt, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of renal cell cancer, in combination with capecitabine, IFN, tamoxifen, IL-2, GM-CSF, Celebrex®, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of gynecologic, uterus or soft tissue sarcoma cancer in combination with IFN, a COX-2 inhibitor such as Celebrex®, and/or sulindac.

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In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of solid tumors in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apicitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with scleroderma or cutaneous vasculitis in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apicitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

This invention also encompasses a method of increasing the dosage of an anti-cancer drug or agent that can be safely and effectively administered to a patient, which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable derivative, salt, solvate, clathrate, hydrate, or pro-drug thereof. Patients that can benefit by this method are those likely to suffer from an adverse effect associated with anti-cancer drugs for treating a specific cancer of the skin, subcutaneous tissue, lymph nodes, brain, lung, liver, bone, intestine, colon, heart, pancreas, adrenal, kidney, prostate, breast, colorectal, or combinations thereof. The administration of an immunomodulatory compound of the invention alleviates or reduces adverse effects which are of such severity that it would otherwise limit the amount of anti-cancer drug.

In one embodiment, an immunomodulatory compound of the invention can be administered orally and daily in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 50 mg, more preferably from about 2 to about 25 mg prior to, during, or after the occurrence of the adverse effect associated with the administration of an anti-cancer drug to a patient. In a particular embodiment, an immunomodulatory compound of the invention is administered in combination with specific agents such as heparin, aspirin, coumadin, or G-CSF to avoid adverse effects that are associated with anti-cancer drugs such as but not limited to neutropenia or thrombocytopenia.

In one embodiment, an immunomodulatory compound of the invention can be administered to patients with diseases and disorders associated with, or characterized by, undesired angiogenesis in combination with additional active ingredients including but not limited to anti-cancer drugs, anti-inflammatories, antihistamines, antibiotics, and steroids.

In another embodiment, this invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with (e.g. before, during, or after) conventional therapy including, but not limited to, surgery, immunotherapy, biological therapy, radiation therapy, or other non-drug based therapy presently used to treat, prevent or manage cancer. The combined use of the immunomodulatory compounds of the invention and conventional therapy may provide a unique treatment regimen that is unexpectedly effective in certain patients. Without being limited by theory, it is believed that immunomodulatory compounds of the invention may provide additive or synergistic effects when given concurrently with conventional therapy.

As discussed elsewhere herein, the invention encompasses a method of reducing, treating and/or preventing adverse or undesired effects associated with conventional therapy including, but not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy. One or more immunomodulatory compounds of the invention and other active ingredient can be administered



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to a patient prior to, during, or after the occurrence of the adverse effect associated with conventional therapy.

In one embodiment, an immunomodulatory compound of the invention can be administered in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 25 mg, more preferably from about 2 to about 10 mg orally and daily alone, or in combination with a second active agent disclosed herein (see, e.g., section 5.2), prior to, during, or after the use of conventional therapy.

In a specific embodiment of this method, an immunomodulatory compound of the invention and doxorubicin are administered to patients with non-small cell lung cancer who were previously treated with carboplatin and radiotherapy.

### 5.3.2 Use with Transplantation Therapy

Compounds of the invention can be used to reduce the risk of Graft Versus Host Disease (GVHD). Therefore, the invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering the immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with transplantation therapy.

As those of ordinary skill in the art are aware, the treatment of cancer is often based on the stages and mechanism of the disease. For example, as inevitable leukemic transformation develops in certain stages of cancer, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. The combined use of the immunomodulatory compound of the invention and transplantation therapy provides a unique and unexpected synergism. In particular, an immunomodulatory compound of the invention exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy in patients with cancer.

An immunomodulatory compound of the invention can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of GVHD. This invention encompasses a method of treating, preventing and/or managing cancer which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after the transplantation of umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow. Examples of stem cells suitable for use in the methods of the invention are disclosed in U.S. provisional patent application No. 60/372,348, filed Apr. 12, 2002 by R. Hariri et al., the entirety of which is incorporated herein by reference.

In one embodiment of this method, an immunomodulatory compound of the invention is administered to patients with multiple myeloma before, during, or after the transplantation of autologous peripheral blood progenitor cell.

In another embodiment, an immunomodulatory compound is administered to patients with relapsing multiple myeloma after the stem cell transplantation.

In another embodiment, an immunomodulatory compound and prednisone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous stem cell.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as salvage therapy for low risk post transplantation to patients with multiple myeloma.

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In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous bone marrow.

In another embodiment, an immunomodulatory compound is administered following the administration of high dose of melphalan and the transplantation of autologous stem cell to patients with chemotherapy responsive multiple myeloma.

In another embodiment, an immunomodulatory compound and PEG INTRO-A are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous CD34-selected peripheral stem cell.

In another embodiment, an immunomodulatory compound is administered with post transplant consolidation chemotherapy to patients with newly diagnosed multiple myeloma to evaluate anti-angiogenesis.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy after DCEP consolidation, following the treatment with high dose of melphalan and the transplantation of peripheral blood stem cell to 65 years of age or older patients with multiple myeloma.

### 5.3.3 Cycling Therapy

In certain embodiments, the prophylactic or therapeutic agents of the invention are cyclically administered to a patient. Cycling therapy involves the administration of an active agent for a period of time, followed by a rest for a period of time, and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

Consequently, in one specific embodiment of the invention, an immunomodulatory compound of the invention is administered daily in a single or divided doses in a four to six week cycle with a rest period of about a week or two weeks. The invention further allows the frequency, number, and length of dosing cycles to be increased. Thus, another specific embodiment of the invention encompasses the administration of an immunomodulatory compound of the invention for more cycles than are typical when it is administered alone. In yet another specific embodiment of the invention, an immunomodulatory compound of the invention is administered for a greater number of cycles that would typically cause dose-limiting toxicity in a patient to whom a second active ingredient is not also being administered.

In one embodiment, an immunomodulatory compound of the invention is administered daily and continuously for three or four weeks at a dose of from about 0.1 to about 150 mg/d followed by a break of one or two weeks. Actimid™ is preferably administered daily and continuously at an initial dose of 0.1 to 5 mg/d with dose escalation (every week) by 1 to 10 mg/d to a maximum dose of 50 mg/d for as long as therapy is tolerated. In a particular embodiment, Revimid™ is administered in an amount of about 5, 10, or 25 mg/day, preferably in an amount of about 10 mg/day for three to four weeks, followed by one week or two weeks of rest in a four or six week cycle.

In one embodiment of the invention, an immunomodulatory compound of the invention and a second active ingredient are administered orally, with administration of an immunomodulatory compound of the invention occurring 30 to 60 minutes prior to a second active ingredient, during a cycle of four to six weeks. In another embodiment of the invention, the combination of an immunomodulatory compound of the

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invention and a second active ingredient is administered by intravenous infusion over about 90 minutes every cycle. In a specific embodiment, one cycle comprises the administration of from about 10 to about 25 mg/day of Revimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient daily for three to four weeks and then one or two weeks of rest. In another specific embodiment, each cycle comprises the administration of from about 5 to about 10 mg/day of Actimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient for 3 to 4 weeks followed by one or two weeks of rest. Typically, the number of cycles during which the combinatorial treatment is administered to a patient will be from about one to about 24 cycles, more typically from about two to about 16 cycles, and even more typically from about four to about three cycles.

#### 5.4 Pharmaceutical Compositions and Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Pharmaceutical compositions and dosage forms of the invention can further comprise one or more excipients.

Pharmaceutical compositions and dosage forms of the invention can also comprise one or more additional active ingredients. Consequently, pharmaceutical compositions and dosage forms of the invention comprise the active ingredients disclosed herein (e.g., an immunomodulatory compound and a second active agent). Examples of optional second, or additional, active ingredients are disclosed herein (see, e.g., section 5.2).

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), topical (e.g., eye drops or other ophthalmic preparations), transdermal or transcutaneous administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; eye drops or other ophthalmic preparations suitable for topical administration; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

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Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients may be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose other mono- or disaccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the *U.S. Pharmacopeia* (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will

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decompose. Such compounds, which are referred to herein as “stabilizers,” include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of from about 0.10 to about 150 mg. Typical dosage forms comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of about 0.1, 1, 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 50, 100, 150 or 200 mg. In a particular embodiment, a preferred dosage form comprises 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) in an amount of about 1, 2, 5, 10, 25 or 50 mg. In a specific embodiment, a preferred dosage form comprises 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) in an amount of about 5, 10, 25 or 50 mg. Typical dosage forms comprise the second active ingredient in an amount of 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. Of course, the specific amount of the anti-cancer drug will depend on the specific agent used, the type of cancer being treated or managed, and the amount(s) of an immunomodulatory compound of the invention and any optional additional active agents concurrently administered to the patient.

#### 5.4.1 Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

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For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil),



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zinc stearate, ethyl oleate, ethyl laurate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

A preferred solid oral dosage form of the invention comprises an immunomodulatory compound of the invention, anhydrous lactose, microcrystalline cellulose, polyvinylpyrrolidone, stearic acid, colloidal anhydrous silica, and gelatin.

#### 5.4.2 Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845, 770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674, 533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

#### 5.4.3 Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous,

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intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention. For example, cyclodextrin and its derivatives can be used to increase the solubility of an immunomodulatory compound of the invention and its derivatives. See, e.g., U.S. Pat. No. 5,134,127, which is incorporated herein by reference.

#### 5.4.4 Topical and Mucosal Dosage Forms

Topical and mucosal dosage forms of the invention include, but are not limited to, sprays, aerosols, solutions, emulsions, suspensions, eye drops or other ophthalmic preparations, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide topical and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form solutions, emulsions or gels, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton Pa. (1980 & 1990).

The pH of a pharmaceutical composition or dosage form may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts,

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hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

#### 5.4.5 Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a dosage form of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, pro-drug, or clathrate thereof. Kits encompassed by this invention can further comprise additional active ingredients such as oblimersen (Genasense®), melphalan, G-CSF, GM-CSF, EPO, topotecan, dacarbazine, irinotecan, taxotere, IFN, COX-2 inhibitor, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13 cis-retinoic acid, or a pharmacologically active mutant or derivative thereof, or a combination thereof. Examples of the additional active ingredients include, but are not limited to, those disclosed herein (see, e.g., section 5.2).

Kits of the invention can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

### 6. EXAMPLES

Certain embodiments of the invention are illustrated by the following non-limiting examples.

#### 6.1 Modulation of Cytokine Production

A series of non-clinical pharmacology and toxicology studies have been performed to support the clinical evaluation of an immunomodulatory compound of the invention in human subjects. These studies were performed in accordance with internationally recognized guidelines for study design and in compliance with the requirements of Good Laboratory Practice (GLP), unless otherwise noted.

Inhibition of TNF- $\alpha$  production following LPS-stimulation of human PBMC and human whole blood by 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Ac-timid™), 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione and thalidomide (Revimid™) was investigated in vitro (Muller et al., *Bioorg. Med. Chem. Lett.* 9:1625-1630, 1999). The IC<sub>50</sub>'s of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and

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human whole blood were ~24 nM (6.55 ng/mL) and ~25 nM (6.83 ng/mL), respectively. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but at least 200 times more potent than, thalidomide. In vitro studies have also demonstrated that concentrations of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione of 2.73 to 27.3 ng/mL (0.01 to 0.1  $\mu$ M) achieved 50% inhibition of the proliferation of MM.1S and Hs Sultan cells.

The IC<sub>50</sub>'s of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and human whole blood were ~100 nM (25.9 ng/mL) and ~480 nM (103.6 ng/mL), respectively. Thalidomide, in contrast, had an IC<sub>50</sub> of ~194  $\mu$ M (50.2  $\mu$ g/mL) for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but 50 to 2000 times more potent than, thalidomide. It has been shown that the compound is approximately 50-100 times more potent than thalidomide in stimulating the proliferation of T-cells following primary induction by T-cell receptor (TCR) activation. 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is also approximately 50 to 100 times more potent than thalidomide in augmenting the production of IL-2 and IFN- $\gamma$  following TCR activation of PBMC (IL-2) or T-cells (IFN- $\gamma$ ). In addition, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione exhibited dose-dependent inhibition of LPS-stimulated production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 by PBMC while it increased production of the anti-inflammatory cytokine IL-10.

#### 6.2 Inhibition of MM Cell Proliferation

The ability of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide for comparison to effect the proliferation of MM cell lines has been investigated in an in vitro study. Uptake [<sup>3</sup>H]-thymidine by different MM cell lines (MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of cell proliferation. Cells were incubated in the presence of compounds for 48 hours; [<sup>3</sup>H]-thymidine was included for the last 8 hours of the incubation period. Addition of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione to MM.1S and Hs Sultan cells resulted in 50% inhibition of cell proliferation at concentrations of 0.4  $\mu$ M and 1  $\mu$ M, respectively. In contrast, addition of thalidomide at concentrations up to 100  $\mu$ M resulted in only 15% and 20% inhibition of cell proliferation in MM.1S and Hs Sultan cells, respectively. These data are summarized in FIG. 1.

#### 6.3 Toxicology Studies

The effects of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) on cardiovascular and respiratory function are investigated in anesthetized dogs. Two groups of Beagle dogs (2/sex/group) are used. One group receives three doses of vehicle only and the other receives three ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (2, 10, and 20 mg/kg). In all cases, doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione or vehicle are successively administered via infusion through the jugular vein separated by intervals of at least 30 minutes.

The cardiovascular and respiratory changes induced by 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione are minimal at all doses when compared to the vehicle control group. The only statistically significant difference between the vehicle and treatment groups is a small

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increase in arterial blood pressure (from 94 mmHg to 101 mmHg) following administration of the low dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. This effect lasts approximately 15 minutes and is not seen at higher doses. Deviations in femoral blood flow, respiratory parameters, and Qtc interval are common to both the control and treated groups and are not considered treatment-related.

#### 6.4 Cycling Therapy in Patients

In a specific embodiment, an immunomodulatory compound of the invention are cyclically administered to patients with cancer. Cycling therapy involves the administration of a first agent for a period of time, followed by a rest for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

In a specific embodiment, prophylactic or therapeutic agents are administered in a cycle of about 4 to 6 weeks, about once or twice every day. One cycle can comprise the administration of a therapeutic on prophylactic agent for three to four weeks and at least a week or two weeks of rest. The number of cycles administered is from about one to about 24 cycles, more typically from about two to about 16 cycles, and more typically from about four to about eight cycles.

For example, in a cycle of four weeks, on day 1, the administration of 25 mg/d of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is started. On day 22, the administration of the compound is stopped for a week of rest. On day 29, the administration of 25 mg/d 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidin-2,6-dione is begun.

#### 6.5 Clinical Studies in Patients

##### 6.5.1 Treatment of Relapsed Multiple Myeloma

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) was administered to patients with relapsed/refractory multiple myeloma. The study was conducted in compliance with Good Clinical Practices. Patients were at least 18 years old, had been diagnosed with multiple myeloma (with paraprotein in serum and/or urine), and were considered refractory to treatment after at least two cycles of treatment, or have relapsed after two cycles of treatment.

Patients who have progressive disease, according to the Southwest Oncology Group (SWOG) criteria, on their prior regimen are considered treatment refractory. Relapse following remission is defined as >25% increase in M component from baseline levels; reappearance of the M paraprotein that had previously disappeared; or a definite increase in the size and number of lytic bone lesions recognized on radiographs. Patients may have had prior therapy with thalidomide, provided they were able to tolerate the treatment. A Zubrod performance status of 0 to 2 is required for all patients.

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione is administered to patients at doses of 1, 2, 5, or 10 mg/day for up to four weeks; at each dose level, three patients are initially enrolled. Dosing occurs at approximately the same time each morning; all doses are administered in the fasted state (no eating for at least two hours prior to dosing and two hours after dosing). 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione doses are administered in an ascending fashion such that patients in the first cohort receive the lowest dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (1 mg/day) and escalation to the next higher dose

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level occurs only following the establishment of safety and tolerability at the current dose. If one out of three patients at any dose level experience dose limiting toxicity (DLT), three additional patients are enrolled at that dose. If none of the three additional patients experience DLT, escalation to the next dose level occurs; dose escalations continue in a similar fashion until the MTD is established or the maximum daily dose (10 mg/day) is attained. However, if one of the three additional patients enrolled experiences DLT, the MTD has been reached. If two or more of the three additional patients enrolled experience DLT, the MTD is judged to have been exceeded and three additional patients are enrolled at the preceding dose level to confirm the MTD. Once the MTD has been identified, four additional patients are enrolled at that dose level so that a total of 10 patients is treated at the MTD.

Blood sampling for analysis of pharmacokinetic parameters is performed on Days 1 and 28 according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. An additional blood sample is collected at each weekly visit for the determination of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione levels. Total urine collections are also made with urine pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Safety assessments are made by monitoring adverse events, vital signs, ECGs, clinical laboratory evaluations (blood chemistry, hematology, lymphocyte phenotyping, and urinalysis), and physical examination at specific times during the study.

Results of interim pharmacokinetic analyses obtained following single- and multiple-dose administration of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione to multiple myeloma patients are presented below in Tables 1 and 2. These data show that 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione was steadily absorbed at all dose levels in relapsed multiple myeloma patients. Maximum plasma concentrations occurred at a median  $T_{max}$  of between 2.5 and 2.8 hours post-dose at Day 1 and between 3 and 4 hours post-dose at Week 4. At all doses, plasma concentrations declined in a monophasic manner after reaching  $C_{max}$ . The start of the elimination phase occurred between 3 and 10 hours post-dose at Day 1 and Week 4, respectively.

These data also showed that after 4 weeks of dosing, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione accumulated to a small extent (mean accumulation ratios ~1.02 to 1.52 and ~0.94 to 1.62 for  $C_{max}$  and  $AUC_{(0-\infty)}$ , respectively). There was almost a dose proportional increase in  $AUC_{(0-\infty)}$  and  $C_{max}$  values with increasing dose. A five-fold higher dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione produced a 3.2- and 2.2-fold increase in  $C_{max}$  at Day 1 and Week 4, respectively. Similarly, a 5-fold increase in dose resulted in a 3.6- and 2.3-fold increase in  $AUC_{(0-\infty)}$ , at Day 1 and Week 4, respectively.

TABLE 1

Pharmacokinetic parameters of Actimid™ in relapsed multiple myeloma patients				
Parameter		1 mg (N = 6)	2 mg (N = 2)	5 mg (N = 3)
Day 1				
$C_{max}$	ng/mL	15.03 (4.04)	24.4* (12.1)	48.56 (14.03)
$t_{max}$	h	3.3 (2.6)	2.7* (0.3)	2.3 (0.3)
$AUC_{(0-\infty)}$	ng · h/mL	152.90 (36.62)	279.18 (51.10)	593.10 (335.23)



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TABLE 1-continued

Pharmacokinetic parameters of Actimid™ in relapsed multiple myeloma patients			
Parameter	1 mg (N = 6)	2 mg (N = 2)	5 mg (N = 3)
AUC <sub>(0-τ)</sub>	134.21 (27.14)	249.57 (29.26)	520.94 (267.32)
t <sub>1/2</sub> h	7.3 (3.4)	6.3 (1.4)	6.5 (2.2)
CL/F mL/min	114.75 (29.20)	121.43 (22.22)	182.31 (117.06)
Vz/f L	69.55 (44.97)	65.31 (2.80)	87.24 (22.61)

τ = 24 hours

N/A = not available

TABLE 2

Pharmacokinetic parameters of Actimid™ following multiple oral doses (1, 2, and 5 mg/day) in relapsed multiple myeloma patients			
Parameter	1 mg (N = 5)	2 mg (N = 2)	5 mg (N = 3)
Week 4			
C <sub>max</sub> ng/mL	23.20 (7.48)	30.05* (15.64)	58.07 (38.08)
t <sub>max</sub> h	3.6 (1.5)	2.8* (0.3)	5.0 (2.6)
AUC <sub>(0-∞)</sub> ng · h/mL	N/A	N/A	N/A
AUC <sub>(0-τ)</sub>	239.31 (122.59)	269.36 (186.34)	597.24 (354.23)
t <sub>1/2</sub> h	6.2* (0.6)	7.7 (2.8)	7.8 (4.0)
CL/F mL/min	87.85 (48.48)	162.68 (112.54)	207.50 (175.41)
Vz/f L	41.35* (8.84)	95.04 (35.39)	103.95 (27.25)

τ = 24 hours

N/A = not available

\*N = 3 patients

### 6.5.2 Treatment of Relapsed Multiple Myeloma

Two Phase 1 clinical studies of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) have been conducted to identify the maximum tolerated dose (MTD) in patients with refractory or relapsed multiple myeloma. These studies have also characterized the safety profile of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione when ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione were given orally for up to 4 weeks. Patients started 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment at 5 mg/day with subsequent escalation to 10, 25, and 50 mg/day. Patients were enrolled for 28 days at their assigned dose, with the option of extended treatment for those who did not exhibit disease progression or experience dose limiting toxicity (DLT). Patients were evaluated for adverse events at each visit and the severity of these events was graded according to the National Cancer Institute (NCI) Common Toxicity Criteria. Patients were discontinued if they experienced DLT (Grade 3 or greater non-hematological, or Grade 4 hematological toxicity).

In this study, 27 patients were enrolled. All patients had relapsed multiple myeloma and 18 (72%) were refractory to salvage therapy. Among these patients, 15 had undergone prior autologous stem cell transplantation and 16 patients had

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received prior thalidomide treatment. The median number of prior regimens was 3 (range 2 to 6).

Blood and urine samples were collected for analysis of pharmacokinetic parameters on Days 1 and 28. Blood samples were collected according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. In addition, a blood sample was collected at each weekly clinic visit for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione determination. Total urine was collected and pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Response to treatment was assessed by M-protein quantification (by immunoelectrophoresis) from serum and a 24-hour urine collection, with creatinine clearance and 24-hour protein calculations undertaken at screening, baseline, Weeks 2 and 4, and monthly thereafter (or upon early termination). Bone marrow aspirations and/or tissue biopsy are also performed at Months 3, 6 and 12 if a patient's paraprotein serum concentration or 24-hour urine protein excretion declined to the next lower level, based on best response criteria. Preliminary results for the 28-day treatment period are summarized below.

Preliminary pharmacokinetic analyses based on these two studies indicated that AUC and C<sub>max</sub> values increase proportionally with dose following single and multiple doses in multiple myeloma patients (as was seen in healthy volunteers). Further, there was no evidence of accumulation with multiple dosing as single dose AUC<sub>(0-∞)</sub> was comparable to multiple dose AUC<sub>(0-τ)</sub> following the same dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Similar to healthy volunteer studies, double peaks were observed. Exposure in multiple myeloma patients appeared to be slightly higher based on C<sub>max</sub> and AUC values as compared to healthy male volunteers while clearance in multiple myeloma patients was lower than it was in healthy volunteers, consistent with their poorer renal function (both as a consequence of their age and their disease). Finally, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione half-life in patients was shorter than in healthy volunteers (mean 8 hours, ranging up to 17 hours).

In this study, the first cohort of 3 patients was treated for 28 days at 5 mg/day without any dose limiting toxicity (DLT). The second cohort of 3 patients subsequently commenced therapy at 10 mg/day. Patients in the second 10 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione cohort tolerated treatment well.

### 6.5.3 Treatment of Solid Tumors

Study with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) was conducted in patients with varying types of solid tumors, including malignant melanoma (13), carcinoma of the pancreas (2), carcinoid-unknown primary (1), renal carcinoma (1), breast carcinoma (1) and NSCLC (2). Patients received 5 mg/day 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for seven days and are subsequently escalated every seven days to 10 mg/day, 25 mg/day, and 50 mg/day for a total of 4 weeks of treatment. Patients who, experienced clinical benefit were permitted to continue on treatment as Named Patients.

The study initially enrolled 20 patients and was subsequently amended to enroll 16 additional patients (adrenal carcinoma, NSCLC, malignant mesothelioma, breast cancer, malignant melanoma (8), renal cell cancer (4)) at a higher dose. The 16 additional patients were given weekly escalating doses of 25 mg/day, 50 mg/day, 75 mg/day, 100 mg/day, 125

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mg/day, and 150 mg/day over a 6-week period with continuing treatment for an additional six weeks.

The study of Phase 1 study was designed to determine a maximum tolerated dose (MTD) of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in patients with refractory solid tumors and/or lymphoma, as well as to characterize the pharmacokinetic and side effect profiles of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in this patient population. The study design dictates that at least 3 patients must be enrolled at a dose level and have completed 28 days of treatment prior to enrollment of patients at the next higher dose level. Patients in the first cohort began dosing at 5 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Patients will be escalated to 10, 20, 25, and 30 mg/day provided there is no toxicity.

In this study, the MTD is defined as the highest dose level in which fewer than two of six patients treated did not experience Grade 3 or greater non-hematological toxicity or Grade 4 or greater hematological toxicity. If, at any given dose level in either study, one out of three patients experiences toxicity, three additional patients must be treated at that particular dose. If, however, two out of six patients experience DLT, the MTD is judged to have been exceeded. No further dose escalations are to occur and additional patients are to be enrolled at the previous dose level. The dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered is escalated until the MTD is achieved or the maximum daily dose of is reached.

No DLTs were reported in the initial group of 20 patients enrolled in the study. Thirteen of the original 20 trial patients, along with 2 non-trial patients, continued on treatment as named patients at doses up to 150 mg/day.

#### 6.5.4 Treatment of Gliomas

This study was performed to find toxicity in patients with recurrent, high-grade gliomas. The study is designed such that patients are given increasingly higher doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione until a maximum tolerated dose (MTD) is established. The study also seeks to obtain preliminary toxicity information and pharmacokinetic data on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, as well as to develop exploratory data concerning surrogate end points of angiogenic activity in vivo using functional neuro-imaging studies, and in vitro assays of serum angiogenic peptides.

Patients enrolled in the first cohort receive 2.5 mg/m<sup>2</sup>/day for a 4-week cycle. During each 4-week cycle of therapy, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered once daily for 3 weeks followed by a week of rest. Patients who complete a treatment cycle may receive another cycle of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment if two criteria are met. First, the patient must have stable disease or have experienced a partial response or complete response, or the patient is benefiting from the therapy with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione as evidenced by a decrease in tumor-related symptoms such as neurological deficits. Second, the patient must have recovered from toxicity related to 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione which occurred in the prior cycle by Day 42 or sooner (28-day cycle plus limit of 2 weeks to recover) as evidenced by a return to Grade  $\leq$ 1 toxicity level. Patients who experience DLT in the previous cycle should have their dose modified. DLT is defined as a non-hematological event Grade  $\geq$ 3 toxicity or hematological event of Grade 4 toxicity thought to be related to the study

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medication. Patients who experience DLT in the first cycle and have no response to therapy are removed from the study.

3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione doses are subsequently escalated to 5, 8, 11, 15, and 20 mg/m<sup>2</sup>/day to a maximum total daily dose of 40 mg. Patients continue to receive 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione on a 4-week cycle per dose level until one of the off-study criteria are met.

Three patients are enrolled in each cohort. If at least one DLT occurs, three additional patients are added to the cohort at that particular dose level. If two DLTs occur, the MTD, defined as the dose at which fewer than one-third of patients at each dose level experiences DLT has been exceeded and four more patients are treated at the previous dose.

Patients who experience DLT during the first 4-week cycle are removed from the study, except if they have a response to therapy. For patients who have completed their first 4-week cycle of without DLT, but who subsequently experience Grade 3 or 4 hematological and/or nonhematological toxicity, treatment is suspended for a minimum of a week. If the toxicity resolves to  $<$ Grade 2 within three weeks, the patient is treated at two dose levels lower than the dose that caused the toxicity (or a 50% reduction if the patient was treated at the first or second dose level). Patients in whom Grade 3 or 4 toxicity does not resolve to  $<$ Grade 1 within three weeks, or those who have another Grade 3 toxicity at the reduced dose are removed from the study.

Pharmacokinetic sampling is performed prior the first dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Day 1) and 0.5, 1, 2, 4, 6, 8, 24, and 48 hours thereafter. Sampling is also conducted pre-dose on Days 7 and 21 and 0.5, 1, 2, 4, 6, 8, and 24 post-dose on Day 21 to evaluate steady-state 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione levels.

#### 6.5.5 Treatment of Metastatic Melanoma

Patients with metastatic melanoma were started on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revmid<sup>TM</sup>) at 5 mg/day for seven days. The dose was then increased every seven days to 10 mg/day, 25 mg/day, and 50 mg/day, respectively, for a total of four weeks on therapy. Five of the 13 melanoma patients who were treated under this regimen either showed disease stabilization or a partial response in the first four weeks of treatment. Tumor response was seen in cutaneous and subcutaneous lesions (five patients), lymph nodes (two patients), and liver (one patient). The duration of response was approximately six months. The result suggests that the compound appears is a promising new anti-cancer agent and has both antiangiogenic and immunomodulatory properties.

#### 6.5.6 Treatment of Relapsed or Refractory Multiple Myeloma

Patients with relapsed and refractory Dune-Salmon stage III multiple myeloma, who have either failed at least three previous regimens or presented with poor performance status, neutropenia or thrombocytopenia, are treated with up to four cycles of combination of melphalan (50 mg intravenously), an immunomodulatory compound of the invention (about 1 to 150 mg orally daily), and dexamethasone (40 mg/day orally on days 1 to 4) every four to six weeks. Maintenance treatment consisting of daily an immunomodulatory compound of the invention and monthly dexamethasone are continued until the disease progression. The therapy using an immunomodulatory compound of the invention in combination with mel-

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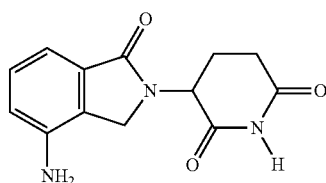
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phalan and dexamethasone is highly active and generally tolerated in heavily pretreated multiple myeloma patients whose prognosis is otherwise poor.

The embodiments of the invention described above are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials, and procedures. All such equivalents are considered to be within the scope of the invention and are encompassed by the appended claims.

What is claimed is:

1. A method of treating multiple myeloma, which comprises administering to a patient having multiple myeloma about 1 to about 50 mg per day of a compound having the formula:



or a pharmaceutically acceptable salt, solvate or stereoisomer thereof, wherein the patient has previously received stem cell transplantation.

2. The method of claim 1, wherein the multiple myeloma is relapsed, refractory, or relapsed and refractory multiple myeloma.

3. The method of claim 1, wherein the compound is administered for 21 consecutive days followed by seven consecutive days of rest in a 28 day cycle.

4. The method of claim 1, wherein the compound is administered every day.

5. The method of claim 1, wherein the compound is administered orally.

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6. The method of claim 1, wherein the compound is administered in the form of a capsule or tablet.

7. The method of claim 1, wherein the compound is administered in a capsule of 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg or 25 mg.

8. The method of claim 6, wherein the capsule comprises the compound, lactose anhydrous, microcrystalline cellulose, croscarmellose sodium and magnesium stearate.

9. The method of claim 1, wherein the compound is administered in an amount of about 25 mg per day.

10. The method of claim 1, wherein the compound is administered in an amount of about 15 mg per day.

11. The method of claim 1, wherein the compound is administered in an amount of about 10 mg per day.

12. The method of claim 1, wherein the compound is administered in an amount of about 5 mg per day.

13. The method of claim 3, wherein the compound is administered in an amount of about 25 mg per day.

14. The method of claim 4, wherein the compound is administered in an amount of about 5 mg, 10 mg or 15 mg per day.

15. The method of claim 1, wherein the stem cell transplantation is autologous stem cell transplantation.

16. The method of claim 1, wherein the stem cell transplantation is hematopoietic stem cell transplantation.

17. The method of claim 1, wherein the stem cell transplantation is peripheral blood stem cell transplantation.

18. The method of claim 1, wherein the compound is administered in an amount of about 2.5 mg per day.

19. The method of claim 3, wherein the compound is administered in an amount of about 10 mg per day.

20. The method of claim 1, wherein the compound is administered in an amount of about 20 mg per day.

21. The method of claim 1, wherein the compound is administered in a capsule containing from about 1 to about 50 mg of the compound.

\* \* \* \* \*



# EXHIBIT J



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(12) **United States Patent**  
**Zeldis**

(10) **Patent No.:** **US 9,101,622 B2**  
(45) **Date of Patent:** **\*Aug. 11, 2015**

(54) **METHODS FOR TREATING NEWLY  
DIAGNOSED MULTIPLE MYELOMA  
3-(4-AMINO-1-OXO-1,3-  
DIHYDRO-ISOINDOL-2-YL)-PIPERIDINE-  
2,6-DIONE IN COMBINATION WITH  
DEXAMETHASONE**

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/482,745**

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**A61K 31/00** (2006.01)  
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**A61K 31/573** (2006.01)  
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**A61K 39/395** (2006.01)  
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CPC ..... **A61K 31/454** (2013.01); **A61K 31/00** (2013.01); **A61K 31/198** (2013.01); **A61K 31/40** (2013.01); **A61K 31/4035** (2013.01); **A61K 31/425** (2013.01); **A61K 31/445** (2013.01); **A61K 31/4439** (2013.01); **A61K 31/475** (2013.01); **A61K 31/515** (2013.01); **A61K 31/573** (2013.01); **A61K 31/675** (2013.01); **A61K 31/704** (2013.01); **A61K 31/7048** (2013.01); **A61K 35/12** (2013.01); **A61K 39/0011** (2013.01); **A61K 39/395** (2013.01); **A61K 45/06** (2013.01)

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See application file for complete search history.

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(57) **ABSTRACT**

Methods of treating, preventing and/or managing cancer as well as and diseases and disorders associated with, or characterized by, undesired angiogenesis are disclosed. Specific methods encompass the administration of an immunomodulatory compound alone or in combination with a second active ingredient. The invention further relates to methods of reducing or avoiding adverse side effects associated with chemotherapy, radiation therapy, hormonal therapy, biological therapy or immunotherapy which comprise the administration of an immunomodulatory compound. Pharmaceutical compositions, single unit dosage forms, and kits suitable for use in methods of the invention are also disclosed.

**25 Claims, 1 Drawing Sheet**

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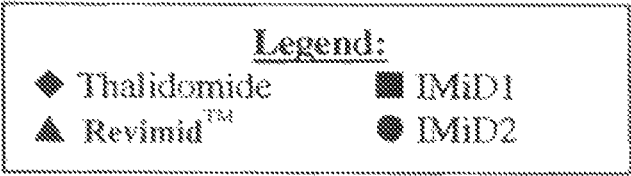
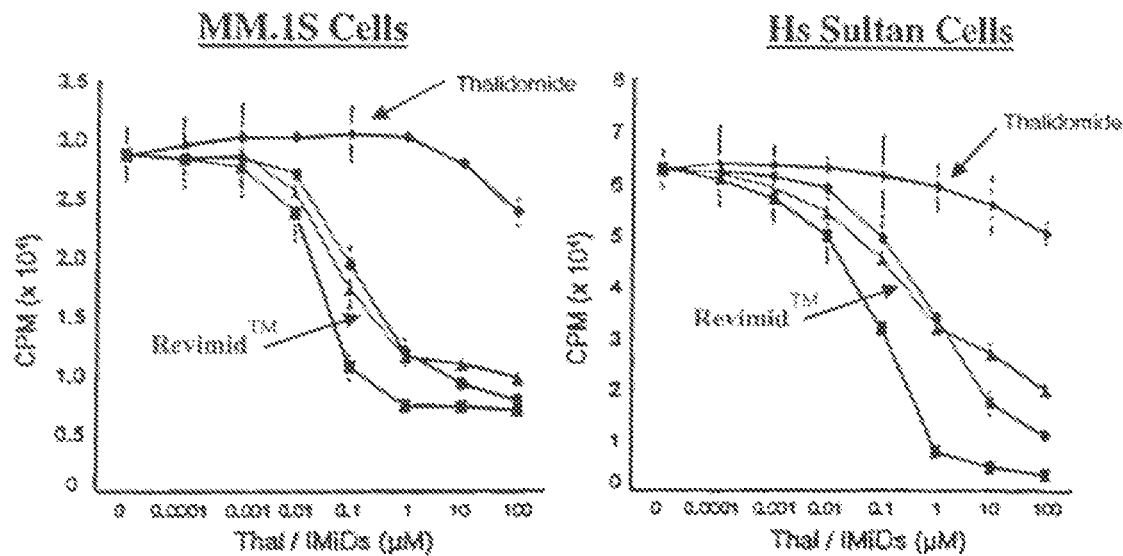
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U.S. Patent

Aug. 11, 2015

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Effects of Revimid™ and Thalidomide on MM Cell Proliferation



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**METHODS FOR TREATING NEWLY  
DIAGNOSED MULTIPLE MYELOMA  
3-(4-AMINO-1-OXO-1,3-DIHYDRO-ISOINDOL-  
2-YL)-PIPERIDINE-2,6-DIONE IN  
COMBINATION WITH DEXAMETHASONE**

This application is a continuation of U.S. patent application Ser. No. 14/255,211, filed Apr. 17, 2014, which is continuation of U.S. patent application Ser. No. 14/201,069, filed Mar. 7, 2014, which is a continuation of U.S. patent application Ser. No. 13/782,728, filed Mar. 1, 2013, now U.S. Pat. No. 8,673,939, which is a continuation of U.S. patent application Ser. No. 13/488,888, filed Jun. 5, 2012, now U.S. Pat. No. 8,648,095, which is a continuation of U.S. patent application Ser. No. 12/640,702, filed Dec. 17, 2009, now U.S. Pat. No. 8,198,306, which is a continuation of U.S. patent application Ser. No. 10/438,213, filed May 15, 2003, now U.S. Pat. No. 7,968,569, which claims the benefit of U.S. provisional application No. 60/380,842, filed May 17, 2002, and 60/424,600, filed Nov. 6, 2002, the entireties of which are incorporated herein by reference.

## 1. FIELD OF THE INVENTION

This invention relates to methods of treating, preventing and/or managing specific cancers, and other diseases including, but not limited to, those associated with, or characterized by, undesired angiogenesis, by the administration of one or more immunomodulatory compounds alone or in combination with other therapeutics. In particular, the invention encompasses the use of specific combinations, or "cocktails," of drugs and other therapy, e.g., radiation to treat these specific cancers, including those refractory to conventional therapy. The invention also relates to pharmaceutical compositions and dosing regimens.

## 2. BACKGROUND OF THE INVENTION

### 2.1 Pathobiology of Cancer and Other Diseases

Cancer is characterized primarily by an increase in the number of abnormal cells derived from a given normal tissue, invasion of adjacent tissues by these abnormal cells, or lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites (metastasis). Clinical data and molecular biologic studies indicate that cancer is a multistep process that begins with minor preneoplastic changes, which may under certain conditions progress to neoplasia. The neoplastic lesion may evolve clonally and develop an increasing capacity for invasion, growth, metastasis, and heterogeneity, especially under conditions in which the neoplastic cells escape the host's immune surveillance. Roitt, I., Brostoff, J and Kale, D., *Immunology*, 17.1-17.12 (3rd ed., Mosby, St. Louis, Mo., 1993).

There is an enormous variety of cancers which are described in detail in the medical literature. Examples includes cancer of the lung, colon, rectum, prostate, breast, brain, and intestine. The incidence of cancer continues to climb as the general population ages, as new cancers develop, and as susceptible populations (e.g., people infected with AIDS or excessively exposed to sunlight) grow. A tremendous demand therefore exists for new methods and compositions that can be used to treat patients with cancer.

Many types of cancers are associated with new blood vessel formation, a process known as angiogenesis. Several of the mechanisms involved in tumor-induced angiogenesis have been elucidated. The most direct of these mechanisms is the secretion by the tumor cells of cytokines with angiogenic

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properties. Examples of these cytokines include acidic and basic fibroblastic growth factor (a,b-FGF), angiogenin, vascular endothelial growth factor (VEGF), and TNF- $\alpha$ . Alternatively, tumor cells can release angiogenic peptides through the production of proteases and the subsequent breakdown of the extracellular matrix where some cytokines are stored (e.g., b-FGF). Angiogenesis can also be induced indirectly through the recruitment of inflammatory cells (particularly macrophages) and their subsequent release of angiogenic cytokines (e.g., TNF- $\alpha$ , bFGF).

A variety of other diseases and disorders are also associated with, or characterized by, undesired angiogenesis. For example, enhanced or unregulated angiogenesis has been implicated in a number of diseases and medical conditions including, but not limited to, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, rubeosis (neovascularization of the angle), viral diseases, genetic diseases, inflammatory diseases, allergic diseases, and autoimmune diseases. Examples of such diseases and conditions include, but are not limited to: diabetic retinopathy; retinopathy of prematurity; corneal graft rejection; neovascular glaucoma; retrolental fibroplasia; and proliferative vitreoretinopathy.

Accordingly, compounds that can control angiogenesis or inhibit the production of certain cytokines, including TNF- $\alpha$ , may be useful in the treatment and prevention of various diseases and conditions.

### 2.2 Methods of Treating Cancer

Current cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient (see, for example, Stockdale, 1998, *Medicine*, vol. 3, Rubenstein and Federman, eds., Chapter 12, Section IV). Recently, cancer therapy could also involve biological therapy or immunotherapy. All of these approaches pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of a patient or may be unacceptable to the patient. Additionally, surgery may not completely remove neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue. Radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent. Although hormonal therapy can be effective, it is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of cancer cells. Biological therapies and immunotherapies are limited in number and may produce side effects such as rashes or swellings, flu-like symptoms, including fever, chills and fatigue, digestive tract problems or allergic reactions.

With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A majority of cancer chemotherapeutics act by inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division. Gilman et al., *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, Tenth Ed. (McGraw Hill, New York).

Despite availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks. Stockdale, *Medicine*, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. 10, 1998. Almost all chemotherapeutic agents are toxic, and chemotherapy causes significant, and often dangerous side effects including severe nausea, bone marrow depression, and immunosuppression. Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular



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chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even if those agents act by different mechanism from those of the drugs used in the specific treatment. This phenomenon is referred to as pleiotropic drug or multidrug resistance. Because of the drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

Other diseases or conditions associated with, or characterized by, undesired angiogenesis are also difficult to treat. However, some compounds such as protamine, heparin and steroids have been proposed to be useful in the treatment of certain specific diseases. Taylor et al., *Nature* 297:307 (1982); Folkman et al., *Science* 221:719 (1983); and U.S. Pat. Nos. 5,001,116 and 4,994,443. Thalidomide and certain derivatives of it have also been proposed for the treatment of such diseases and conditions. U.S. Pat. Nos. 5,593,990, 5,629,327, 5,712,291, 6,071,948 and 6,114,355 to D'Amato.

Still, there is a significant need for safe and effective methods of treating, preventing and managing cancer and other diseases and conditions, particularly for diseases that are refractory to standard treatments, such as surgery, radiation therapy, chemotherapy and hormonal therapy, while reducing or avoiding the toxicities and/or side effects associated with the conventional therapies.

### 2.3 IMiDS™

A number of studies have been conducted with the aim of providing compounds that can safely and effectively be used to treat diseases associated with abnormal production of TNF- $\alpha$ . See, e.g., Marriott, J. B., et al., *Expert Opin. Biol. Ther.* 1(4):1-8 (2001); G. W. Muller, et al., *Journal of Medicinal Chemistry* 39(17): 3238-3240 (1996); and G. W. Muller, et al., *Bioorganic & Medicinal Chemistry Letters* 8: 2669-2674 (1998). Some studies have focused on a group of compounds selected for their capacity to potentially inhibit TNF- $\alpha$  production by LPS stimulated PBMC. L. G. Corral, et al., *Ann. Rheum. Dis.* 58:(Suppl 1) 1107-1113 (1999). These compounds, which are referred to as IMiDS™ (Celgene Corporation) or Immunomodulatory Drugs, show not only potent inhibition of TNF- $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  and IL12 production. LPS induced IL6 is also inhibited by immunomodulatory compounds, albeit partially. These compounds are potent stimulators of LPS induced IL10. Id. Particular examples of IMiDS™s include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles described in U.S. Pat. Nos. 6,281,230 and 6,316,471, both to G. W. Muller, et al.

### 3. SUMMARY OF THE INVENTION

This invention encompasses methods of treating and preventing certain types of cancer, including primary and metastatic cancer, as well as cancers that are refractory or resistant to conventional chemotherapy. The methods comprise administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. The invention also encompasses methods of managing certain cancers (e.g., preventing or prolonging their recurrence, or lengthening the time of remission) which comprise administering to a patient in need of such management a prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

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In particular methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage cancer. Examples of such conventional therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention also encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are associated with, or characterized by, undesired angiogenesis, which comprise administering to a patient in need of such treatment, management or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In other methods of the invention, an immunomodulatory compound is administered in combination with a therapy conventionally used to treat, prevent or manage diseases or disorders associated with, or characterized by, undesired angiogenesis. Examples of such conventional therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy.

This invention encompasses pharmaceutical compositions, single unit dosage forms, dosing regimens and kits which comprise an immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second, or additional, active agent. Second active agents include specific combinations, or "cocktails," of drugs.

### 4. BRIEF DESCRIPTION OF FIGURE

FIG. 1 shows a comparison of the effects of 3-(4-amino-1-oxo-1,3-dihydro-isindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide in inhibiting the proliferation of multiple myeloma (MM) cell lines in an in vitro study. The uptake of [<sup>3</sup>H]-thymidine by different MM cell lines (MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of the cell proliferation.

### 5. DETAILED DESCRIPTION OF THE INVENTION

A first embodiment of the invention encompasses methods of treating, managing, or preventing cancer which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with another drug ("second active agent") or method of treating, managing, or preventing cancer. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage cancer.

Another embodiment of the invention encompasses methods of treating, managing or preventing diseases and disorders other than cancer that are characterized by undesired angiogenesis. These methods comprise the administration of a therapeutically or prophylactically effective amount of an

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immunomodulatory compound, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof.

Examples of diseases and disorders associated with, or characterized by, undesired angiogenesis include, but are not limited to, inflammatory diseases, autoimmune diseases, viral diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroidal neovascular diseases, retina neovascular diseases, and rubeosis (neovascularization of the angle).

In particular methods encompassed by this embodiment, the immunomodulatory compound is administered in combination with a second active agent or method of treating, managing, or preventing the disease or condition. Second active agents include small molecules and large molecules (e.g., proteins and antibodies), examples of which are provided herein, as well as stem cells. Methods, or therapies, that can be used in combination with the administration of the immunomodulatory compound include, but are not limited to, surgery, blood transfusions, immunotherapy, biological therapy, radiation therapy, and other non-drug based therapies presently used to treat, prevent or manage disease and conditions associated with, or characterized by, undesired angiogenesis.

The invention also encompasses pharmaceutical compositions (e.g., single unit dosage forms) that can be used in methods disclosed herein. Particular pharmaceutical compositions comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, and a second active agent.

#### 5.1 Immunomodulatory Compounds

Compounds used in the invention include immunomodulatory compounds that are racemic, stereomerically enriched or stereomerically pure, and pharmaceutically acceptable salts, solvates, hydrates, stereoisomers, clathrates, and prodrugs thereof. Preferred compounds used in the invention are small organic molecules having a molecular weight less than about 1,000 g/mol, and are not proteins, peptides, oligonucleotides, oligosaccharides or other macromolecules.

As used herein and unless otherwise indicated, the terms “immunomodulatory compounds” and “IMiDs™” (Celgene Corporation) encompasses small organic molecules that markedly inhibit TNF- $\alpha$ , LPS induced monocyte IL1 $\beta$  and IL12, and partially inhibit IL6 production. Specific immunomodulatory compounds are discussed below.

TNF- $\alpha$  is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation. TNF- $\alpha$  is responsible for a diverse range of signaling events within cells. TNF- $\alpha$  may play a pathological role in cancer. Without being limited by theory, one of the biological effects exerted by the immunomodulatory compounds of the invention is the reduction of synthesis of TNF- $\alpha$ . Immunomodulatory compounds of the invention enhance the degradation of TNF- $\alpha$  mRNA.

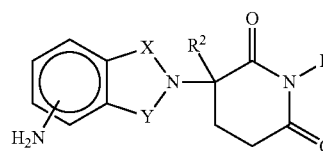
Further, without being limited by theory, immunomodulatory compounds used in the invention may also be potent co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. Immunomodulatory compounds of the invention may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset. In addition, the compounds preferably have anti-inflammatory properties, and efficiently co-stimulate T cells.

Specific examples of immunomodulatory compounds of the invention, include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-

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fluoropiperidin-3-yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl) isoindolines such as those described in U.S. Pat. No. 5,874,448; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolines described in U.S. Pat. No. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines (e.g., 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517; and a class of non-polypeptide cyclic amides disclosed in U.S. Pat. Nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S. Pat. Nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles such as those described in U.S. Pat. Nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those described in U.S. patent application Ser. No. 09/972,487 filed on Oct. 5, 2001, U.S. patent application Ser. No. 10/032,286 filed on Dec. 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents and patent applications identified herein are incorporated herein by reference. Immunomodulatory compounds of the invention do not include thalidomide.

Other specific immunomodulatory compounds of the invention include, but are not limited to, 1-oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein by reference. These compounds have the structure I:



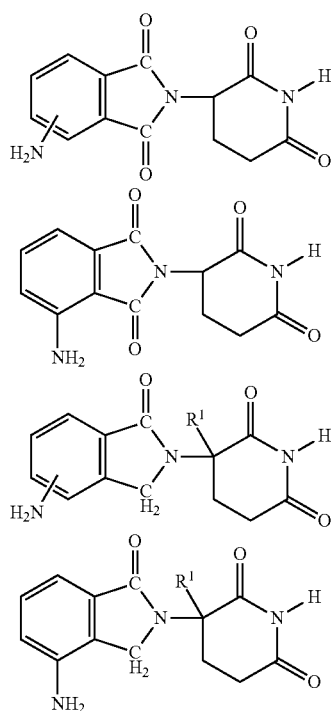
in which one of X and Y is C=O, the other of X and Y is C=O or CH<sub>2</sub>, and R<sup>2</sup> is hydrogen or lower alkyl, in particular methyl. Specific immunomodulatory compounds include, but are not limited to:

- 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;
- 1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;
- 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;
- 1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;
- 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;
- and
- 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

Other specific immunomodulatory compounds of the invention belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles, such as those described in U.S. Pat. Nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (International Publication No. WO 98/03502), each of which is incorporated herein by reference. Compounds representative of this class are of the formulas:

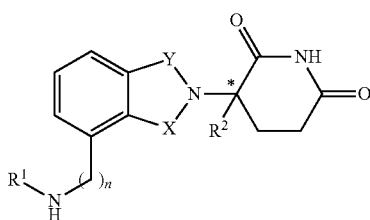
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wherein  $R^1$  is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (e.g. optically pure (R) or (S) enantiomers) of these compounds.

Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. Nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are incorporated herein by reference. Representative compounds are of formula II:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is  $C=O$  and the other is  $CH_2$  or  $C=O$ ;

$R^1$  is H,  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $C(O)R^3$ ,  $C(S)R^3$ ,  $C(O)OR^4$ ,  $(C_1-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $C(O)NHR^3$ ,  $C(S)NHR^3$ ,  $C(O)NR^3R^3$ ,  $C(S)NR^3R^3$  or  $(C_1-C_8)$ alkyl- $O(CO)R^5$ ;

$R^2$  is H, F, benzyl,  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl, or  $(C_2-C_8)$ alkynyl;

$R^3$  and  $R^3'$  are independently  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-$

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$C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_0-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $(C_1-C_8)$ alkyl- $O(CO)R^5$ , or  $C(O)OR^5$ ;

$R^4$  is  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl,  $(C_1-C_4)$ alkyl- $OR^5$ , benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl, or  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl;

$R^5$  is  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl, or  $(C_2-C_5)$ heteroaryl;

each occurrence of  $R^6$  is independently H,  $(C_1-C_8)$ alkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_2-C_5)$ heteroaryl, or  $(C_0-C_8)$ alkyl- $C(O)O-R^5$  or the  $R^6$  groups can join to form a heterocycloalkyl group;

n is 0 or 1; and

\* represents a chiral-carbon center.

In specific compounds of formula II, when n is 0 then  $R^1$  is  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $C(O)R^3$ ,  $C(O)OR^4$ ,  $(C_1-C_8)$ alkyl- $N(R^6)_2$ ,  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $C(S)NHR^3$ , or  $(C_1-C_8)$ alkyl- $O(CO)R^5$ ;

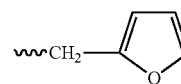
$R^2$  is H or  $(C_1-C_8)$ alkyl; and

$R^3$  is  $(C_1-C_8)$ alkyl,  $(C_3-C_7)$ cycloalkyl,  $(C_2-C_8)$ alkenyl,  $(C_2-C_8)$ alkynyl, benzyl, aryl,  $(C_0-C_4)$ alkyl- $(C_1-C_6)$ heterocycloalkyl,  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_5-C_8)$ alkyl- $N(R^6)_2$ ;  $(C_0-C_8)$ alkyl- $NH-C(O)O-R^5$ ;  $(C_1-C_8)$ alkyl- $OR^5$ ,  $(C_1-C_8)$ alkyl- $C(O)OR^5$ ,  $(C_1-C_8)$ alkyl- $O(CO)R^5$ , or  $C(O)OR^5$ ; and the other variables have the same definitions.

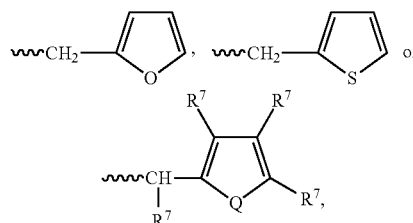
In other specific compounds of formula II,  $R^2$  is H or  $(C_1-C_4)$ alkyl.

In other specific compounds of formula II,  $R^1$  is  $(C_1-C_8)$ alkyl or benzyl.

In other specific compounds of formula II,  $R^1$  is H,  $(C_1-C_8)$ alkyl, benzyl,  $CH_2OCH_3$ ,  $CH_2CH_2OCH_3$ , or



In another embodiment of the compounds of formula II,  $R^1$  is



wherein Q is O or S, and each occurrence of  $R^7$  is independently H,  $(C_1-C_8)$ alkyl, benzyl,  $CH_2OCH_3$ , or  $CH_2CH_2OCH_3$ .

In other specific compounds of formula II,  $R^1$  is  $C(O)R^3$ .

In other specific compounds of formula II,  $R^3$  is  $(C_0-C_4)$ alkyl- $(C_2-C_5)$ heteroaryl,  $(C_1-C_8)$ alkyl, aryl, or  $(C_0-C_4)$ alkyl- $OR^5$ .

In other specific compounds of formula II, heteroaryl is pyridyl, furyl, or thienyl.

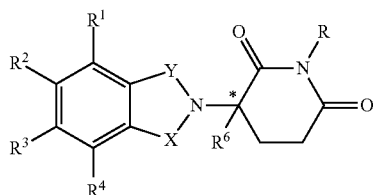
In other specific compounds of formula II,  $R^1$  is  $C(O)OR^4$ .

In other specific compounds of formula II, the H of  $C(O)NHC(O)$  can be replaced with  $(C_1-C_4)$ alkyl, aryl, or benzyl.

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Still other specific immunomodulatory compounds of the invention belong to a class of isoindole-imides disclosed in U.S. patent application Ser. No. 09/781,179, International Publication No. WO 98/54170, and U.S. Pat. No. 6,395,754, each of which are incorporated herein by reference. Representative compounds are of formula III:



and pharmaceutically acceptable salts, hydrates, solvates, clathrates, enantiomers, diastereomers, racemates, and mixtures of stereoisomers thereof, wherein:

one of X and Y is C=O and the other is CH<sub>2</sub> or C=O;

R is H or CH<sub>2</sub>OCOR';

(i) each of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup>, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> is nitro or —NHR<sup>5</sup> and the remaining of R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, or R<sup>4</sup> are hydrogen;

R<sup>5</sup> is hydrogen or alkyl of 1 to 8 carbons

R<sup>6</sup> hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro;

R' is R<sup>7</sup>—CHR<sup>10</sup>—N(R<sup>8</sup>R<sup>9</sup>);

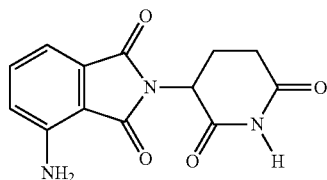
R<sup>7</sup> is m-phenylene or p-phenylene or —(C<sub>n</sub>H<sub>2n</sub>)— in which n has a value of 0 to 4;

each of R<sup>8</sup> and R<sup>9</sup> taken independently of the other is hydrogen or alkyl of 1 to 8 carbon atoms, or R<sup>8</sup> and R<sup>9</sup> taken together are tetramethylene, pentamethylene, hexamethylene, or —CH<sub>2</sub>CH<sub>2</sub>[X]<sub>1</sub>CH<sub>2</sub>CH<sub>2</sub>— in which [X]<sub>1</sub> is —O—, —S—, or —NH—;

R<sup>10</sup> is hydrogen, alkyl of 1 to 8 carbon atoms, or phenyl; and

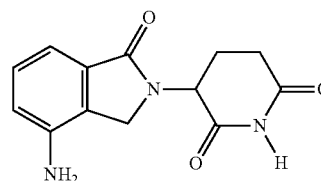
\* represents a chiral-carbon center.

The most preferred immunomodulatory compounds of the invention are 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. The compounds can be obtained via standard, synthetic methods (see e.g., U.S. Pat. No. 5,635, 517, incorporated herein by reference). The compounds are available from Celgene Corporation, Warren, N.J. 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (ACTIMID™) has the following chemical structure:



The compound 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (REVIMID™) has the following chemical structure:

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Compounds of the invention can either be commercially purchased or prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry techniques.

As used herein and unless otherwise indicated, the term “pharmaceutically acceptable salt” encompasses non-toxic acid and base addition salts of the compound to which the term refers. Acceptable non-toxic acid addition salts include those derived from organic and inorganic acids or bases known in the art, which include, for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methane-sulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embolic acid, enanthic acid, and the like.

Compounds that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds are those that form non-toxic base addition salts, i.e., salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzyl-ethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), lysine, and procaine.

As used herein and unless otherwise indicated, the term “prodrug” means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives of immunomodulatory compounds of the invention that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of immunomodulatory compounds of the invention that comprise —NO, —NO<sub>2</sub>, —ONO, or —ONO<sub>2</sub> moieties. Prodrugs can typically be prepared using well-known methods, such as those described in 1 *Burger's Medicinal Chemistry and Drug Discovery*, 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995), and *Design of Prodrugs* (H. Bundgaard ed., Elsevier, N.Y. 1985).

As used herein and unless otherwise indicated, the terms “biohydrolyzable amide,” “biohydrolyzable ester,” “biohydrolyzable carbamate,” “biohydrolyzable carbonate,” “biohydrolyzable ureide,” “biohydrolyzable phosphate” mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically



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active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, lower acyloxyalkyl esters (such as acetoxymethyl, acetoxylethyl, aminocarbonyloxymethyl, pivaloyloxymethyl, and pivaloyloxyethyl esters), lactonyl esters (such as phthalidyl and thiophthalidyl esters), lower alkoxyacyloxyalkyl esters (such as methoxycarbonyloxymethyl, ethoxycarbonyloxyethyl and isopropoxycarbonyloxyethyl esters), alkoxyalkyl esters, choline esters, and acylamino alkyl esters (such as acetamidomethyl esters). Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides,  $\alpha$ -amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, amino acids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

Various immunomodulatory compounds of the invention contain one or more chiral centers, and can exist as racemic mixtures of enantiomers or mixtures of diastereomers. This invention encompasses the use of stereomerically pure forms of such compounds, as well as the use of mixtures of those forms. For example, mixtures comprising equal or unequal amounts of the enantiomers of a particular immunomodulatory compounds of the invention may be used in methods and compositions of the invention. These isomers may be asymmetrically synthesized or resolved using standard techniques such as chiral columns or chiral resolving agents. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. As used herein and unless otherwise indicated, the term "stereomerically enriched" means a composition that comprises greater than about 60% by weight of one stereoisomer of a compound, preferably greater than about 70% by weight, more preferably greater than about 80% by weight of one stereoisomer of a compound. As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center. Similarly, the term "stereomerically enriched" means a stereomerically enriched composition of a compound having one chiral center.

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It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

## 5.2 Second Active Agents

Immunomodulatory compounds can be combined with other pharmacologically active compounds ("second active agents") in methods and compositions of the invention. It is believed that certain combinations work synergistically in the treatment of particular types of cancer and certain diseases and conditions associated with, or characterized by, undesired angiogenesis. Immunomodulatory compounds can also work to alleviate adverse effects associated with certain second active agents, and some second active agents can be used to alleviate adverse effects associated with immunomodulatory compounds.

One or more second active ingredients or agents can be used in the methods and compositions of the invention together with an immunomodulatory compound. Second active agents can be large molecules (e.g., proteins) or small molecules (e.g., synthetic inorganic, organometallic, or organic molecules).

Examples of large molecule active agents include, but are not limited to, hematopoietic growth factors, cytokines, and monoclonal and polyclonal antibodies. Typical large molecule active agents are biological molecules, such as naturally occurring or artificially made proteins. Proteins that are particularly useful in this invention include proteins that stimulate the survival and/or proliferation of hematopoietic precursor cells and immunologically active poietic cells in vitro or in vivo. Others stimulate the division and differentiation of committed erythroid progenitors in cells in vitro or in vivo. Particular proteins include, but are not limited to: interleukins, such as IL-2 (including recombinant IL-II ("rIL2") and canarypox IL-2), IL-10, IL-12, and IL-18; interferons, such as interferon alfa-2a, interferon alfa-2b, interferon alfa-n1, interferon alfa-n3, interferon beta-1 a, and interferon gamma-I b; GM-CSF and GM-CSF; and EPO.

Particular proteins that can be used in the methods and compositions of the invention include, but are not limited to: filgrastim, which is sold in the United States under the trade name Neupogen® (Amgen, Thousand Oaks, Calif.); sargramostim, which is sold in the United States under the trade name Leukine® (Immunex, Seattle, Wash.); and recombinant EPO, which is sold in the United States under the trade name Epogen® (Amgen, Thousand Oaks, Calif.).

Recombinant and mutated forms of GM-CSF can be prepared as described in U.S. Pat. Nos. 5,391,485; 5,393,870; and 5,229,496; all of which are incorporated herein by reference. Recombinant and mutated forms of G-CSF can be prepared as described in U.S. Pat. Nos. 4,810,643; 4,999,291; 5,528,823; and 5,580,755; all of which are incorporated herein by reference.

This invention encompasses the use of native, naturally occurring, and recombinant proteins. The invention further encompasses mutants and derivatives (e.g., modified forms) of naturally occurring proteins that exhibit, in vivo, at least some of the pharmacological activity of the proteins upon which they are based. Examples of mutants include, but are not limited to, proteins that have one or more amino acid residues that differ from the corresponding residues in the naturally occurring forms of the proteins. Also encompassed by the term "mutants" are proteins that lack carbohydrate moieties normally present in their naturally occurring forms

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(e.g., nonglycosylated forms). Examples of derivatives include, but are not limited to, pegylated derivatives and fusion proteins, such as proteins formed by fusing IgG1 or IgG3 to the protein or active portion of the protein of interest. See, e.g., Penichet, M. L. and Morrison, S. L., *J. Immunol. Methods* 248:91-101 (2001).

Antibodies that can be used in combination with compounds of the invention include monoclonal and polyclonal antibodies. Examples of antibodies include, but are not limited to, trastuzumab (Herceptin®), rituximab (Rituxan®), bevacizumab (Avastin™), pertuzumab (Omnitarg™), tositumomab (Bexxar®), edrelomab (Panorex®), and G250. Compounds of the invention can also be combined with, or used in combination with, anti-TNF- $\alpha$  antibodies.

Large molecule active agents may be administered in the form of anti-cancer vaccines. For example, vaccines that secrete, or cause the secretion of, cytokines such as IL-2, G-CSF, and GM-CSF can be used in the methods, pharmaceutical compositions, and kits of the invention. See, e.g., Emens, L. A., et al., *Curr. Opinion Mol. Ther.* 3(1):77-84 (2001).

In one embodiment of the invention, the large molecule active agent reduces, eliminates, or prevents an adverse effect associated with the administration of an immunomodulatory compound. Depending on the particular immunomodulatory compound and the disease or disorder being treated, adverse effects can include, but are not limited to, drowsiness and somnolence, dizziness and orthostatic hypotension, neutropenia, infections that result from neutropenia, increased HIV-viral load, bradycardia, Stevens-Johnson Syndrome and toxic epidermal necrolysis, and seizures (e.g., grand mal convulsions). A specific adverse effect is neutropenia.

Second active agents that are small molecules can also be used to alleviate adverse effects associated with the administration of an immunomodulatory compound. However, like some large molecules, many are believed to be capable of providing a synergistic effect when administered with (e.g., before, after or simultaneously) an immunomodulatory compound. Examples of small molecule second active agents include, but are not limited to, anti-cancer agents, antibiotics, immunosuppressive agents, and steroids.

Examples of anti-cancer agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; celecoxib (COX-2 inhibitor); chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epiropropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofofosine; iproplatin; irinotecan; irinotecan

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hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprostol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procabazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiro-mustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; taxotere; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; testosterone; triceribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; and zorubicin hydrochloride.

Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamcstane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin ITT derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betacalamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; capecitabine; carboxamide-amino-triazole; carboxamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroquinoline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conage-nin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatin; cypemycin; cytarabine ocfosfate; cytolytic factor; cytosatin; dacliximab; decitabine; dehydrotidemnin B; deslorelin; dexamethasone; dexifosfamide; dextrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; doxorubicin; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride;



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estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flzelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofofene; ilomastat; imatinib (e.g., Gleevec®), imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; mcnogaril; merbarone; meterclin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; Erbitux, human chorionic gonadotrophin; monophosphoryl lipid A+mycobacterium cell wall sk; mopidamol; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullin; oblimersen (Genasense®); O<sup>6</sup>-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; penterozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porrimer sodium; porfirofomycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin

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1; squalamine; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; velaresol; veramine; verdins; verteporfin; vinorelbine; vinoxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Specific second active agents include, but are not limited to, oblimersen (Genasense®), remicade, docetaxel, celecoxib, melphalan, dexamethasone (Decadron®), steroids, gemcitabine, cisplatin, temozolomide, etoposide, cyclophosphamide, temodar, carboplatin, procarbazine, gliadel, tamoxifen, topotecan, methotrexate, Arisa®, taxol, taxotere, fluorouracil, leucovorin, irinotecan, xeloda, CPT-11, interferon alpha, pegylated interferon alpha (e.g., PEG INTRON-A), capecitabine, cisplatin, thiotepa, fludarabine, carboplatin, liposomal daunorubicin, cytarabine, doxorubicin, paclitaxel, vinblastine, IL-2, GM-CSF, dacarbazine, vinorelbine, zoledronic acid, palmitronate, biaxin, busulphan, prednisone, bisphosphonate, arsenic trioxide, vincristine, doxorubicin (Doxil®), paclitaxel, ganciclovir, adriamycin, estramustine sodium phosphate (Emcyt®), sulindac, and etoposide.

### 5.3 Methods of Treatments and Prevention

Methods of this invention encompass methods of treating, preventing and/or managing various types of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis. As used herein, unless otherwise specified, the term “treating” refers to the administration of a compound of the invention or other additional active agent after the onset of symptoms of the particular disease or disorder. As used herein, unless otherwise specified, the term “preventing” refers to the administration prior to the onset of symptoms, particularly to patients at risk of cancer, and other diseases and disorders associated with, or characterized by, undesired angiogenesis. The term “prevention” includes the inhibition of a symptom of the particular disease or disorder. Patients with familial history of cancer and diseases and disorders associated with, or characterized by, undesired angiogenesis are preferred candidates for preventive regimens. As used herein and unless otherwise indicated, the term “managing” encompasses preventing the recurrence of the particular disease or disorder in a patient who had suffered from it, and/or lengthening the time a patient who had suffered from the disease or disorder remains in remission.

As used herein, the term “cancer” includes, but is not limited to, solid tumors and blood born tumors. The term “cancer” refers to disease of skin tissues, organs, blood, and vessels, including, but not limited to, cancers of the bladder, bone or blood, brain, breast, cervix, chest, colon, endometrium, esophagus, eye, head, kidney, liver, lymph nodes, lung, mouth, neck, ovaries, pancreas, prostate, rectum, stomach, testis, throat, and uterus. Specific cancers include, but are not limited to, advanced malignancy, amyloidosis, neuroblastoma, meningioma, hemangiopericytoma, multiple brain metastase, glioblastoma multiforms, glioblastoma, brain stem glioma, poor prognosis malignant brain tumor,

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malignant glioma, recurrent malignant glioma, anaplastic astrocytoma, anaplastic oligodendroglioma, neuroendocrine tumor, rectal adenocarcinoma, Dukes C & D colorectal cancer, unresectable colorectal carcinoma, metastatic hepatocellular carcinoma, Kaposi's sarcoma, karotype acute myeloblastic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma, low grade follicular lymphoma, malignant melanoma, malignant mesothelioma, malignant pleural effusion mesothelioma syndrome, peritoneal carcinoma, papillary serous carcinoma, gynecologic sarcoma, soft tissue sarcoma, scleroderma, cutaneous vasculitis, Langerhans cell histiocytosis, leiomyosarcoma, fibrodysplasia ossificans progressive, hormone refractory prostate cancer, resected high-risk soft tissue sarcoma, unresectable hepatocellular carcinoma, Waldenstrom's macroglobulinemia, smoldering myeloma, indolent myeloma, fallopian tube cancer, androgen independent prostate cancer, androgen dependent stage IV non-metastatic prostate cancer, hormone-insensitive prostate cancer, chemotherapy-insensitive prostate cancer, papillary thyroid carcinoma, follicular thyroid carcinoma, medullary thyroid carcinoma, and leiomyoma. In a specific embodiment, the cancer is metastatic. In another embodiment, the cancer is refractory or resistance to chemotherapy or radiation; in particular, refractory to thalidomide.

As used herein to refer to diseases and conditions other than cancer, the terms "diseases or disorders associated with, or characterized by, undesired angiogenesis," "diseases or disorders associated with undesired angiogenesis," and "diseases or disorders characterized by undesired angiogenesis" refer to diseases, disorders and conditions that are caused, mediated or attended by undesired, unwanted or uncontrolled angiogenesis, including, but not limited to, inflammatory diseases, autoimmune diseases, genetic diseases, allergic diseases, bacterial diseases, ocular neovascular diseases, choroidal neovascular diseases, and retina neovascular diseases.

Examples of such diseases or disorders associated with undesired angiogenesis include, but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, proliferative vitreoretinopathy, trachoma, myopia, optic pits, epidemic keratoconjunctivitis, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, syphilis, lipid degeneration, bacterial ulcer, fungal ulcer, Herpes simplex infection, Herpes zoster infection, protozoan infection, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, peripheral radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Beheet's disease, retinitis, choroiditis, presumed ocular histoplasmosis, Bests disease, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, rubeosis, sarcoidosis, sclerosis, soriatis, psoriasis, primary sclerosing cholangitis, proctitis, primary biliary srosis, idiopathic pulmonary fibrosis, and alcoholic hepatitis.

In specific embodiments of the invention, diseases or disorders associated with undesired angiogenesis do not include congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, myocardial infarction, HIV, hepatitis, adult respiratory distress syndrome, bone-resorption disease, chronic obstructive pulmonary diseases, chronic pul-

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monary inflammatory disease, dermatitis, cystic fibrosis, septic shock, sepsis, endotoxic shock, hemodynamic shock, sepsis syndrome, post ischemic reperfusion injury, meningitis, psoriasis, fibrotic disease, cachexia, graft rejection, rheumatoid spondylitis, osteoporosis, Crohn's disease, ulcerative colitis, inflammatory-bowel disease, multiple sclerosis, systemic lupus erythematosus, erythema nodosum leprosum in leprosy, radiation damage, asthma, hyperoxic alveolar injury, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

This invention encompasses methods of treating patients who have been previously treated for cancer or diseases or disorders associated with, or characterized by, undesired angiogenesis, but are non-responsive to standard therapies, as well as those who have not previously been treated. The invention also encompasses methods of treating patients regardless of patient's age, although some diseases or disorders are more common in certain age groups. The invention further encompasses methods of treating patients who have undergone surgery in an attempt to treat the disease or condition at issue, as well as those who have not. Because patients with cancer and diseases and disorders characterized by undesired angiogenesis have heterogenous clinical manifestations and varying clinical outcomes, the treatment given to a patient may vary, depending on his/her prognosis. The skilled clinician will be able to readily determine without undue experimentation specific secondary agents, types of surgery, and types of non-drug based standard therapy that can be effectively used to treat an individual patient with cancer and other diseases or disorders.

Methods encompassed by this invention comprise administering one or more immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, to a patient (e.g., a human) suffering, or likely to suffer, from cancer or a disease or disorder mediated by undesired angiogenesis.

In one embodiment of the invention, an immunomodulatory compound of the invention can be administered orally in single or divided daily doses in an amount of from about 0.10 to about 150 mg/day. In a particular embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of from about 0.1 to about 1 mg per day, or alternatively from about 0.1 to about 5 mg every other day. In a preferred embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered in an amount of from about 5 to 25 mg per day, or alternatively from about 10 to about 50 mg every other day.

In a specific embodiment, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) may be administered in an amount of about 1, 2, or 5 mg per day to patients with relapsed multiple myeloma. In a particular embodiment, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) may be administered initially in an amount of 5 mg/day and the dose can be escalated every week to 10, 20, 25, 30 and 50 mg/day. In a specific embodiment, Revimid™ can be administered in an amount of up to about 30 mg/day to patients with solid tumor. In a particular embodiment, Revimid™ can be administered in an amount of up to about 40 mg/day to patients with glioma.

#### 5.3.1 Combination Therapy with a Second Active Agent

Specific methods of the invention comprise administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in combination with one or more second active agents, and/or in combination with radia-

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tion therapy, blood transfusions, or surgery. Examples of immunomodulatory compounds of the invention are disclosed herein (see, e.g., section 5.1). Examples of second active agents are also disclosed herein (see, e.g., section 5.2).

Administration of the immunomodulatory compounds and the second active agents to a patient can occur simultaneously or sequentially by the same or different routes of administration. The suitability of a particular route of administration employed for a particular active agent will depend on the active agent itself (e.g., whether it can be administered orally without decomposing prior to entering the blood stream) and the disease being treated. A preferred route of administration for an immunomodulatory compound of the invention is orally. Preferred routes of administration for the second active agents or ingredients of the invention are known to those of ordinary skill in the art. See, e.g., *Physicians' Desk Reference*, 1755-1760 (56<sup>th</sup> ed., 2002).

In one embodiment of the invention, the second active agent is administered intravenously or subcutaneously and once or twice daily in an amount of from about 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. The specific amount of the second active agent will depend on the specific agent used, the type of disease being treated or managed, the severity and stage of disease, and the amount(s) of immunomodulatory compounds of the invention and any optional additional active agents concurrently administered to the patient. In a particular embodiment, the second active agent is oblimersen (Genasense®), GM-CSF, G-CSF, EPO, taxotere, irinotecan, dacarbazine, transretinoic acid, topotecan, pentoxifylline, ciprofloxacin, dexamethasone, vincristine, doxorubicin, COX-2 inhibitor, IL2, IL8, IL18, IFN, Ara-C, vinorelbine, or a combination thereof.

In a particular embodiment, GM-CSF, G-CSF or EPO is administered subcutaneously during about five days in a four or six week cycle in an amount of from about 1 to about 750 mg/m<sup>2</sup>/day, preferably in an amount of from about 25 to about 500 mg/m<sup>2</sup>/day, more preferably in an amount of from about 50 to about 250 mg/m<sup>2</sup>/day, and most preferably in an amount of from about 50 to about 200 mg/m<sup>2</sup>/day. In a certain embodiment, GM-CSF may be administered in an amount of from about 60 to about 500 mcg/m<sup>2</sup> intravenously over 2 hours, or from about 5 to about 12 mcg/m<sup>2</sup>/day subcutaneously. In a specific embodiment, G-CSF may be administered subcutaneously in an amount of about 1 mcg/kg/day initially and can be adjusted depending on rise of total granulocyte counts. The maintenance dose of G-CSF may be administered in an amount of about 300 (in smaller patients) or 480 mcg subcutaneously. In a certain embodiment, EPO may be administered subcutaneously in an amount of 10,000 Unit 3 times per week.

In another embodiment, Revimid™ in an amount of about 25 mg/d and dacarbazine in an amount of about from 200 to 1,000 mg/m<sup>2</sup>/d are administered to patients with metastatic malignant melanoma. In a specific embodiment, Revimid™ is administered in an amount of from about 5 to about 25 mg/d to patients with metastatic malignant melanoma whose disease has progressed on treatment with dacarbazine, IL-2 or IFN. In a specific embodiment, Revimid™ is administered to patients with relapsed or refractory multiple myeloma in an amount of about 15 mg/d twice a day or about 30 mg/d four times a day in a combination with dexamethasone.

In another embodiment, an immunomodulatory compound is administered with melphalan and dexamethasone to patients with amyloidosis. In a specific embodiment, an immunomodulatory compound of the invention and steroids can be administered to patients with amyloidosis.

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In another embodiment, an immunomodulatory compound is administered with gemcitabine and cisplatin to patients with locally advanced or metastatic transitional cell bladder cancer.

In another embodiment, an immunomodulatory compound is administered in combination with a second active ingredient as follows: temozolomide to pediatric patients with relapsed or progressive brain tumors or recurrent neuroblastoma; celecoxib, etoposide and cyclophosphamide for relapsed or progressive CNS cancer; temodar to patients with recurrent or progressive meningioma, malignant meningioma, hemangiopericytoma, multiple brain metastases, relapsed brain tumors, or newly diagnosed glioblastoma multiformis; irinotecan to patients with recurrent glioblastoma; carboplatin to pediatric patients with brain stem glioma; procarbazine to pediatric patients with progressive malignant gliomas; cyclophosphamide to patients with poor prognosis malignant brain tumors, newly diagnosed or recurrent glioblastoma multiformis; Gliadel® for high grade recurrent malignant gliomas; temozolomide and tamoxifen for anaplastic astrocytoma; or topotecan for gliomas, glioblastoma, anaplastic astrocytoma or anaplastic oligodendroglioma.

In another embodiment, an immunomodulatory compound is administered with methotrexate and cyclophosphamide to patients with metastatic breast cancer.

In another embodiment, an immunomodulatory compound is administered with temozolomide to patients with neuroendocrine tumors.

In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with recurrent or metastatic head or neck cancer. In another embodiment, an immunomodulatory compound is administered with gemcitabine to patients with pancreatic cancer.

In another embodiment, an immunomodulatory compound is administered to patients with colon cancer in combination with Arisa®, taxol and/or taxotere.

In another embodiment, an immunomodulatory compound is administered with capecitabine to patients with refractory colorectal cancer or patients who fail first line therapy or have poor performance in colon or rectal adenocarcinoma.

In another embodiment, an immunomodulatory compound is administered in combination with fluorouracil, leucovorin, and irinotecan to patients with Dukes C & D colorectal cancer or to patients who have been previously treated for metastatic colorectal cancer.

In another embodiment, an immunomodulatory compound is administered to patients with refractory colorectal cancer in combination with capecitabine, xeloda, and/or CPT-11.

In another embodiment, an immunomodulatory compound of the invention is administered with capecitabine and irinotecan to patients with refractory colorectal cancer or to patients with unresectable or metastatic colorectal carcinoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with interferon alpha or capecitabine to patients with unresectable or metastatic hepatocellular carcinoma; or with cisplatin and thiopeta to patients with primary or metastatic liver cancer.

In another embodiment, an immunomodulatory compound is administered in combination with pegylated interferon alpha to patients with Kaposi's sarcoma.

In another embodiment, an immunomodulatory compound is administered in combination with fludarabine, carboplatin, and/or topotecan to patients with refractory or relapsed or high-risk acuted myelogenous leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with liposomal daunorubicin,



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topotecan and/or cytarabine to patients with unfavorable karyotype acute myeloblastic leukemia.

In another embodiment, an immunomodulatory compound is administered in combination with gemcitabine and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered in combination with carboplatin and irinotecan to patients with non-small cell lung cancer. In one embodiment, an immunomodulatory compound is administered with doctaxol to patients with non-small cell lung cancer who have been previously treated with carbo/VP 16 and radiotherapy.

In another embodiment, an immunomodulatory compound is administered in combination with carboplatin and/or taxotere, or in combination with carboplatin, paclitaxel and/or thoracic radiotherapy to patients with non-small cell lung cancer. In a specific embodiment, an immunomodulatory compound is administered in combination with taxotere to patients with stage IIIB or TV non-small cell lung cancer.

In another embodiment, an immunomodulatory compound of the invention is administered in combination with oblimersen (Genasense®) to patients with small cell lung cancer.

In another embodiment, an immunomodulatory compound is administered alone or in combination with a second active ingredient such as vinblastine or fludarabine to patients with various types of lymphoma, including, but not limited to, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cutaneous T-Cell lymphoma, cutaneous B-Cell lymphoma, diffuse large B-Cell lymphoma or relapsed or refractory low grade follicular lymphoma.

In another embodiment, an immunomodulatory compound is administered in combination with taxotere, IL-2, IFN, GM-CSF, and/or dacarbazine to patients with various types or stages of melanoma.

In another embodiment, an immunomodulatory compound is administered alone or in combination with vinorelbine to patients with malignant mesothelioma, or stage IIIB non-small cell lung cancer with pleural implants or malignant pleural effusion mesothelioma syndrome.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of multiple myeloma in combination with dexamethasone, zoledronic acid, palmitronate, GM-CSF, biacin, vinblastine, melphalan, busulphan, cyclophosphamide, IFN, palmidronate, prednisone, bisphosphonate, celecoxib, arsenic trioxide, PEG INTRON-A, vincristine, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with relapsed or refractory multiple myeloma in combination with doxorubicin (Doxil®), vincristine and/or dexamethasone (Decadron®).

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of ovarian cancer such as peritoneal carcinoma, papillary serous carcinoma, refractory ovarian cancer or recurrent ovarian cancer, in combination with taxol, carboplatin, doxorubicin, gemcitabine, cisplatin, xeloda, paclitaxel, dexamethasone, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of prostate cancer, in combination with xeloda, 5 FU/LV, gemcitabine, irinotecan plus gemcitabine, cyclophosphamide, vincristine, dexamethasone, GM-CSF, celecoxib, taxotere, ganciclovir, paclitaxel, adriamycin, docetaxel, estramustine, Emcyt, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of

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renal cell cancer, in combination with capecitabine, IFN, tamoxifen, IL-2, GM-CSF, Celebrex®, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of gynecologic, uterus or soft tissue sarcoma cancer in combination with IFN, a COX-2 inhibitor such as Celebrex®, and/or sulindac.

In another embodiment, an immunomodulatory compound is administered to patients with various types or stages of solid tumors in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apecitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

In another embodiment, an immunomodulatory compound is administered to patients with scleroderma or cutaneous vasculitis in combination with celebrex, etoposide, cyclophosphamide, docetaxel, apecitabine, IFN, tamoxifen, IL-2, GM-CSF, or a combination thereof.

This invention also encompasses a method of increasing the dosage of an anti-cancer drug or agent that can be safely and effectively administered to a patient, which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable derivative, salt, solvate, clathrate, hydrate, or prodrug thereof. Patients that can benefit by this method are those likely to suffer from an adverse effect associated with anti-cancer drugs for treating a specific cancer of the skin, subcutaneous tissue, lymph nodes, brain, lung, liver, bone, intestine, colon, heart, pancreas, adrenal, kidney, prostate, breast, colorectal, or combinations thereof. The administration of an immunomodulatory compound of the invention alleviates or reduces adverse effects which are of such severity that it would otherwise limit the amount of anti-cancer drug.

In one embodiment, an immunomodulatory compound of the invention can be administered orally and daily in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 50 mg, more preferably from about 2 to about 25 mg prior to, during, or after the occurrence of the adverse effect associated with the administration of an anti-cancer drug to a patient. In a particular embodiment, an immunomodulatory compound of the invention is administered in combination with specific agents such as heparin, aspirin, coumadin, or G-CSF to avoid adverse effects that are associated with anti-cancer drugs such as but not limited to neutropenia or thrombocytopenia.

In one embodiment, an immunomodulatory compound of the invention can be administered to patients with diseases and disorders associated with, or characterized by, undesired angiogenesis in combination with additional active ingredients including but not limited to anti-cancer drugs, anti-inflammatories, antihistamines, antibiotics, and steroids.

In another embodiment, this invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with (e.g. before, during, or after) conventional therapy including, but not limited to, surgery, immunotherapy, biological therapy, radiation therapy, or other non-drug based therapy presently used to treat, prevent or manage cancer. The combined use of the immunomodulatory compounds of the invention and conventional therapy may provide a unique treatment regimen that is unexpectedly effective in certain patients. Without being limited by theory, it is believed that immunomodulatory compounds of the invention may provide additive or synergistic effects when given concurrently with conventional therapy.

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As discussed elsewhere herein, the invention encompasses a method of reducing, treating and/or preventing adverse or undesired effects associated with conventional therapy including, but not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, biological therapy and immunotherapy. One or more immunomodulatory compounds of the invention and other active ingredient can be administered to a patient prior to, during, or after the occurrence of the adverse effect associated with conventional therapy.

In one embodiment, an immunomodulatory compound of the invention can be administered in an amount of from about 0.1 to about 150 mg, and preferably from about 1 to about 25 mg, more preferably from about 2 to about 10 mg orally and daily alone, or in combination with a second active agent disclosed herein (see, e.g., section 5.2), prior to, during, or after the use of conventional therapy.

In a specific embodiment of this method, an immunomodulatory compound of the invention and doxorubicin are administered to patients with non-small cell lung cancer who were previously treated with carboplatin and radiotherapy.

### 5.3.2 Use with Transplantation Therapy

Compounds of the invention can be used to reduce the risk of Graft Versus Host Disease (GVHD). Therefore, the invention encompasses a method of treating, preventing and/or managing cancer, which comprises administering the immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, in conjunction with transplantation therapy.

As those of ordinary skill in the art are aware, the treatment of cancer is often based on the stages and mechanism of the disease. For example, as inevitable leukemic transformation develops in certain stages of cancer, transplantation of peripheral blood stem cells, hematopoietic stem cell preparation or bone marrow may be necessary. The combined use of the immunomodulatory compound of the invention and transplantation therapy provides a unique and unexpected synergism. In particular, an immunomodulatory compound of the invention exhibits immunomodulatory activity that may provide additive or synergistic effects when given concurrently with transplantation therapy in patients with cancer.

An immunomodulatory compound of the invention can work in combination with transplantation therapy reducing complications associated with the invasive procedure of transplantation and risk of GVHD. This invention encompasses a method of treating, preventing and/or managing cancer which comprises administering to a patient (e.g., a human) an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof, before, during, or after the transplantation of umbilical cord blood, placental blood, peripheral blood stem cell, hematopoietic stem cell preparation or bone marrow. Examples of stem cells suitable for use in the methods of the invention are disclosed in U.S. provisional patent application No. 60/372,348, filed Apr. 12, 2002 by R. Hariri et al., the entirety of which is incorporated herein by reference.

In one embodiment of this method, an immunomodulatory compound of the invention is administered to patients with multiple myeloma before, during, or after the transplantation of autologous peripheral blood progenitor cell.

In another embodiment, an immunomodulatory compound is administered to patients with relapsing multiple myeloma after the stem cell transplantation.

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In another embodiment, an immunomodulatory compound and prednisone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous stem cell.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as salvage therapy for low risk post transplantation to patients with multiple myeloma.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous bone marrow.

In another embodiment, an immunomodulatory compound is administered following the administration of high dose of melphalan and the transplantation of autologous stem cell to patients with chemotherapy responsive multiple myeloma.

In another embodiment, an immunomodulatory compound and PEG INTRO-A are administered as maintenance therapy to patients with multiple myeloma following the transplantation of autologous CD34-selected peripheral stem cell.

In another embodiment, an immunomodulatory compound is administered with post transplant consolidation chemotherapy to patients with newly diagnosed multiple myeloma to evaluate anti-angiogenesis.

In another embodiment, an immunomodulatory compound and dexamethasone are administered as maintenance therapy after DCEP consolidation, following the treatment with high dose of melphalan and the transplantation of peripheral blood stem cell to 65 years of age or older patients with multiple myeloma.

### 5.3.3 Cycling Therapy

In certain embodiments, the prophylactic or therapeutic agents of the invention are cyclically administered to a patient. Cycling therapy involves the administration of an active agent for a period of time, followed by a rest for a period of time, and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

Consequently, in one specific embodiment of the invention, an immunomodulatory compound of the invention is administered daily in a single or divided doses in a four to six week cycle with a rest period of about a week or two weeks. The invention further allows the frequency, number, and length of dosing cycles to be increased. Thus, another specific embodiment of the invention encompasses the administration of an immunomodulatory compound of the invention for more cycles than are typical when it is administered alone. In yet another specific embodiment of the invention, an immunomodulatory compound of the invention is administered for a greater number of cycles that would typically cause dose-limiting toxicity in a patient to whom a second active ingredient is not also being administered.

In one embodiment, an immunomodulatory compound of the invention is administered daily and continuously for three or four weeks at a dose of from about 0.1 to about 150 mg/d followed by a break of one or two weeks. Actimid™ is preferably administered daily and continuously at an initial dose of 0.1 to 5 mg/d with dose escalation (every week) by 1 to 10 mg/d to a maximum dose of 50 mg/d for as long as therapy is tolerated. In a particular embodiment, Revimid™ is administered in an amount of about 5, 10, or 25 mg/day, preferably in an amount of about 10 mg/day for three to four weeks, followed by one week or two weeks of rest in a four or six week cycle.

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In one embodiment of the invention, an immunomodulatory compound of the invention and a second active ingredient are administered orally, with administration of an immunomodulatory compound of the invention occurring 30 to 60 minutes prior to a second active ingredient, during a cycle of four to six weeks. In another embodiment of the invention, the combination of an immunomodulatory compound of the invention and a second active ingredient is administered by intravenous infusion over about 90 minutes every cycle. In a specific embodiment, one cycle comprises the administration of from about 10 to about 25 mg/day of Revimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient daily for three to four weeks and then one or two weeks of rest. In another specific embodiment, each cycle comprises the administration of from about 5 to about 10 mg/day of Actimid™ and from about 50 to about 200 mg/m<sup>2</sup>/day of a second active ingredient for 3 to 4 weeks followed by one or two weeks of rest. Typically, the number of cycles during which the combinatorial treatment is administered to a patient will be from about one to about 24 cycles, more typically from about two to about 16 cycles, and even more typically from about four to about three cycles.

#### 5.4 Pharmaceutical Compositions and Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms of the invention comprise an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Pharmaceutical compositions and dosage forms of the invention can further comprise one or more excipients.

Pharmaceutical compositions and dosage forms of the invention can also comprise one or more additional active ingredients. Consequently, pharmaceutical compositions and dosage forms of the invention comprise the active ingredients disclosed herein (e.g., an immunomodulatory compound and a second active agent). Examples of optional second, or additional, active ingredients are disclosed herein (see, e.g., section 5.2).

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), topical (e.g., eye drops or other ophthalmic preparations), transdermal or transcutaneous administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; eye drops or other ophthalmic preparations suitable for topical administration; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease. These and other ways in which

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specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton, Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients may be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose other mono- or disaccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the *U.S. Pharmacopeia* (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples



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of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as “stabilizers,” include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of from about 0.10 to about 150 mg. Typical dosage forms comprise an immunomodulatory compound of the invention or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof in an amount of about 0.1, 1, 2, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 50, 100, 150 or 200 mg. In a particular embodiment, a preferred dosage form comprises 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) in an amount of about 1, 2, 5, 10, 25 or 50 mg. In a specific embodiment, a preferred dosage form comprises 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) in an amount of about 5, 10, 25 or 50 mg. Typical dosage forms comprise the second active ingredient in an amount of 1 to about 1000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg, or from about 50 to about 200 mg. Of course, the specific amount of the anti-cancer drug will depend on the specific agent used, the type of cancer being treated or managed, and the amount(s) of an immunomodulatory compound of the invention and any optional additional active agents concurrently administered to the patient.

#### 5.4.1 Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton, Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and inti-

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ately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other alginates, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene

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glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W. R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

A preferred solid oral dosage form of the invention comprises an immunomodulatory compound of the invention, anhydrous lactose, microcrystalline cellulose, polyvinylpyrrolidone, stearic acid, colloidal anhydrous silica, and gelatin.

#### 5.4.2 Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

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#### 5.4.3 Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention. For example, cyclodextrin and its derivatives can be used to increase the solubility of an immunomodulatory compound of the invention and its derivatives. See, e.g., U.S. Pat. No. 5,134,127, which is incorporated herein by reference.

#### 5.4.4 Topical and Mucosal Dosage Forms

Topical and mucosal dosage forms of the invention include, but are not limited to, sprays, aerosols, solutions, emulsions, suspensions, eye drops or other ophthalmic preparations, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton, Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide topical and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form solutions, emulsions or gels, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> and 18<sup>th</sup> eds., Mack Publishing, Easton, Pa. (1980 & 1990).

The pH of a pharmaceutical composition or dosage form may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-

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enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

#### 5.4.5 Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a dosage form of an immunomodulatory compound of the invention, or a pharmaceutically acceptable salt salt, solvate, hydrate, stereoisomer, prodrug, or clathrate thereof. Kits encompassed by this invention can further comprise additional active ingredients such as oblimersen (Genasense®), melphalan, G-CSF, GM-CSF, EPO, topotecan, dacarbazine, irinotecan, taxotere, IFN, COX-2 inhibitor, pentoxifylline, ciprofloxacin, dexamethasone, IL2, IL8, IL18, Ara-C, vinorelbine, isotretinoin, 13 cis-retinoic acid, or a pharmacologically active mutant or derivative thereof, or a combination thereof. Examples of the additional active ingredients include, but are not limited to, those disclosed herein (see, e.g., section 5.2).

Kits of the invention can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise cells or blood for transplantation as well as pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

## 6. EXAMPLES

Certain embodiments of the invention are illustrated by the following non-limiting examples.

### 6.1 Modulation of Cytokine Production

A series of non-clinical pharmacology and toxicology studies have been performed to support the clinical evaluation of an immunomodulatory compound of the invention in human subjects. These studies were performed in accordance with internationally recognized guidelines for study design and in compliance with the requirements of Good Laboratory Practice (GLP), unless otherwise noted.

Inhibition of TNF- $\alpha$  production following LPS-stimulation of human PBMC and human whole blood by 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Ac-timid™), 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione and thalidomide (Revimid™) was investigated in vitro (Muller et al., *Bioorg. Med. Chem. Lett.* 9:1625-1630, 1999). The IC<sub>50</sub>'s of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and human whole blood were ~24 nM (6.55 ng/mL) and ~25 nM (6.83 ng/mL), respectively. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-

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isoindol-2-yl)-piperidine-2,6-dione that is similar to, but at least 200 times more potent than, thalidomide. In vitro studies have also demonstrated that concentrations of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione of 2.73 to 27.3 ng/mL (0.01 to 0.1  $\mu$ M) achieved 50% inhibition of the proliferation of MM.1S and Hs Sultan cells.

The IC<sub>50</sub>'s of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC and human whole blood were ~100 nM (25.9 ng/mL) and ~480 nM (103.6 ng/mL), respectively. Thalidomide, in contrast, had an IC<sub>50</sub> of ~194  $\mu$ M (50.2  $\mu$ g/mL) for inhibiting production of TNF- $\alpha$  following LPS-stimulation of PBMC. In vitro studies suggest a pharmacological activity profile for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione that is similar to, but 50 to 2000 times more potent than, thalidomide. It has been shown that the compound is approximately 50-100 times more potent than thalidomide in stimulating the proliferation of T-cells following primary induction by T-cell receptor (TCR) activation. 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is also approximately 50 to 100 times more potent than thalidomide in augmenting the production of IL-2 and IFN- $\gamma$  following TCR activation of PBMC (IL-2) or T-cells (IFN- $\gamma$ ). In addition, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione exhibited dose-dependent inhibition of LPS-stimulated production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 by PBMC while it increased production of the anti-inflammatory cytokine IL-10.

### 6.2 Inhibition of MM Cell Proliferation

The ability of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) and thalidomide for comparison to effect the proliferation of MM cell lines has been investigated in an in vitro study. Uptake [<sup>3</sup>H]-thymidine by different MM cell lines MM.1S, Hs Sultan, U266 and RPMI-8226) was measured as an indicator of cell proliferation. Cells were incubated in the presence of compounds for 48 hours; [<sup>3</sup>H]-thymidine was included for the last 8 hours of the incubation period. Addition of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione to MM.1S and Hs Sultan cells resulted in 50% inhibition of cell proliferation at concentrations of 0.4  $\mu$ M and 1  $\mu$ M, respectively. In contrast, addition of thalidomide at concentrations up to 100  $\mu$ M resulted in only 15% and 20% inhibition of cell proliferation in MM.1S and Hs Sultan cells, respectively. These data are summarized in FIG. 1.

### 6.3 Toxicology Studies

The effects of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) on cardiovascular and respiratory function are investigated in anesthetized dogs. Two groups of Beagle dogs (2/sex/group) are used. One group receives three doses of vehicle only and the other receives three ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (2, 10, and 20 mg/kg). In all cases, doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione or vehicle are successively administered via infusion through the jugular vein separated by intervals of at least 30 minutes.

The cardiovascular and respiratory changes induced by 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione are minimal at all doses when compared to the vehicle control group. The only statistically significant difference between the vehicle and treatment groups is a small increase in arterial blood pressure (from 94 mmHg to 101 mmHg) following administration of the low dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. This effect lasts approximately 15 minutes and is not seen at higher doses. Deviations in femoral blood flow, res-



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piratory parameters, and Qtc interval are common to both the control and treated groups and are not considered treatment-related.

#### 6.4 Cycling Therapy in Patients

In a specific embodiment, an immunomodulatory compound of the invention are cyclically administered to patients with cancer. Cycling therapy involves the administration of a first agent for a period of time, followed by a rest for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improves the efficacy of the treatment.

In a specific embodiment, prophylactic or therapeutic agents are administered in a cycle of about 4 to 6 weeks, about once or twice every day. One cycle can comprise the administration of a therapeutic or prophylactic agent for three to four weeks and at least a week or two weeks of rest. The number of cycles administered is from about one to about 24 cycles, more typically from about two to about 16 cycles, and more typically from about four to about eight cycles.

For example, in a cycle of four weeks, on day 1, the administration of 25 mg/d of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is started. On day 22, the administration of the compound is stopped for a week of rest. On day 29, the administration of 25 mg/d 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione is begun.

#### 6.5 Clinical Studies in Patients

##### 6.5.1 Treatment of Relapsed Multiple Myeloma

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (Actimid™) was administered to patients with relapsed/refractory multiple myeloma. The study was conducted in compliance with Good Clinical Practices. Patients were at least 18 years old, had been diagnosed with multiple myeloma (with paraprotein in serum and/or urine), and were considered refractory to treatment after at least two cycles of treatment, or have relapsed after two cycles of treatment.

Patients who have progressive disease, according to the Southwest Oncology Group (SWOG) criteria, on their prior regimen are considered treatment refractory. Relapse following remission is defined as >25% increase in M component from baseline levels; reappearance of the M paraprotein that had previously disappeared; or a definite increase in the size and number of lytic bone lesions recognized on radiographs. Patients may have had prior therapy with thalidomide, provided they were able to tolerate the treatment. A Zubrod performance status of 0 to 2 is required for all patients.

4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione is administered to patients at doses of 1, 2, 5, or 10 mg/day for up to four weeks; at each dose level, three patients are initially enrolled. Dosing occurs at approximately the same time each morning; all doses are administered in the fasted state (no eating for at least two hours prior to dosing and two hours after dosing). 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione doses are administered in an ascending fashion such that patients in the first cohort receive the lowest

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dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (1 mg/day) and escalation to the next higher dose level occurs only following the establishment of safety and tolerability at the current dose. If one out of three patients at any dose level experience dose limiting toxicity (DLT), three additional patients are enrolled at that dose. If none of the three additional patients experience DLT, escalation to the next dose level occurs; dose escalations continue in a similar fashion until the MTD is established or the maximum daily dose (10 mg/day) is attained. However, if one of the three additional patients enrolled experiences DLT, the MTD has been reached. If two or more of the three additional patients enrolled experience DLT, the MTD is judged to have been exceeded and three additional patients are enrolled at the preceding dose level to confirm the MTD. Once the MTD has been identified, four additional patients are enrolled at that dose level so that a total of 10 patients is treated at the MTD.

Blood sampling for analysis of pharmacokinetic parameters is performed on Days 1 and 28 according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. An additional blood sample is collected at each weekly visit for the determination of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione levels. Total urine collections are also made with urine pooled according to the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Safety assessments are made by monitoring adverse events, vital signs, ECGs, clinical laboratory evaluations (blood chemistry, hematology, lymphocyte phenotyping, and urinalysis), and physical examination at specific times during the study.

Results of interim pharmacokinetic analyses obtained following single- and multiple-dose administration of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione to multiple myeloma patients are presented below in Tables 1 and 2. These data show that 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione was steadily absorbed at all dose levels in relapsed multiple myeloma patients. Maximum plasma concentrations occurred at a median  $T_{max}$  of between 2.5 and 2.8 hours post-dose at Day 1 and between 3 and 4 hours post-dose at Week 4. At all doses, plasma concentrations declined in a monophasic manner after reaching  $C_{max}$ . The start of the elimination phase occurred between 3 and 10 hours post-dose at Day 1 and Week 4, respectively.

These data also showed that after 4 weeks of dosing, 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione accumulated to a small extent (mean accumulation ratios ~1.02 to 1.52 and ~0.94 to 1.62 for  $C_{max}$  and  $AUC_{(0-\infty)}$ , respectively). There was almost a dose proportional increase in  $AUC_{(0-\infty)}$  and  $C_{max}$  values with increasing dose. A five-fold higher dose of 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione produced a 3.2- and 2.2-fold increase in  $C_{max}$  at Day 1 and Week 4, respectively. Similarly, a 5-fold increase in dose resulted in a 3.6- and 2.3-fold increase in  $AUC_{(0-\infty)}$ , at Day 1 and Week 4, respectively.

TABLE 1

Pharmacokinetic parameters of Actimid™ in relapsed multiple myeloma patients				
Parameter		1 mg (N = 6)	2 mg (N = 2)	5 mg (N = 3)
Day 1				
$C_{max}$	ng/mL	15.03 (4.04)	24.4* (12.1)	48.56 (14.03)
$t_{max}$	h	3.3 (2.6)	2.7* (0.3)	2.3 (0.3)
$AUC_{(0-\infty)}$	ng · h/mL	152.90 (36.62)	279.18 (51.10)	593.10 (335.23)

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TABLE 1-continued

Pharmacokinetic parameters of Actimid™ in relapsed multiple myeloma patients				
Parameter		1 mg (N = 6)	2 mg (N = 2)	5 mg (N = 3)
AUC <sub>(0-τ)</sub>		134.21 (27.14)	249.57 (29.26)	520.94 (267.32)
t <sub>1/2</sub>	h	7.3 (3.4)	6.3 (1.4)	6.5 (2.2)
CL/F	mL/min	114.75 (29.20)	121.43 (22.22)	182.31 (117.06)
Vz/f	L	69.55 (44.97)	65.31 (2.80)	87.24 (22.61)

τ = 24 hours

N/A = not available

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TABLE 2

Pharmacokinetic parameters of Actimid™ following multiple oral doses (1, 2, and 5 mg/day) in relapsed multiple myeloma patients				
Parameter		1 mg (N = 5)	2 mg (N = 2)	5 mg (N = 3)
Week 4				
C <sub>max</sub>	ng/mL	23.20 (7.48)	30.05* (15.64)	58.07 (38.08)
t <sub>max</sub>	h	3.6 (1.5)	2.8* (0.3)	5.0 (2.6)
AUC <sub>(0-∞)</sub>	ng · h/mL	N/A	N/A	N/A
AUC <sub>(0-τ)</sub>		239.31 (122.59)	269.36 (186.34)	597.24 (354.23)
t <sub>1/2</sub>	h	6.2* (0.6)	7.7 (2.8)	7.8 (4.0)
CL/F	mL/min	87.85 (48.48)	162.68 (112.54)	207.50 (175.41)
Vz/f	L	41.35* (8.84)	95.04 (35.39)	103.95 (27.25)

τ = 24 hours

N/A = not available

\*N = 3 patients

### 6.5.2 Treatment of Relapsed Multiple Myeloma

Two Phase 1 clinical studies of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) have been conducted to identify the maximum tolerated dose (MTD) in patients with refractory or relapsed multiple myeloma. These studies have also characterized the safety profile of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione when ascending doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione were given orally for up to 4 weeks. Patients started 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment at 5 mg/day with subsequent escalation to 10, 25, and 50 mg/day. Patients were enrolled for 28 days at their assigned dose, with the option of extended treatment for those who did not exhibit disease progression or experience dose limiting toxicity (DLT). Patients were evaluated for adverse events at each visit and the severity of these events was graded according to the National Cancer Institute (NCI) Common Toxicity Criteria. Patients were discontinued if they experienced DLT (Grade 3 or greater non-hematological, or Grade 4 hematological toxicity).

In this study, 27 patients were enrolled. All patients had relapsed multiple myeloma and 18 (72%) were refractory to salvage therapy. Among these patients, 15 had undergone prior autologous stem cell transplantation and 16 patients had received prior thalidomide treatment. The median number of prior regimens was 3 (range 2 to 6).

Blood and urine samples were collected for analysis of pharmacokinetic parameters on Days 1 and 28. Blood samples were collected according to the following sampling schedule: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose. In addition, a blood sample was collected at each weekly clinic visit for 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione determination. Total urine was collected and pooled according to

the following time intervals post-dose: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Response to treatment was assessed by M-protein quantification (by immunoelectrophoresis) from serum and a 24-hour urine collection, with creatinine clearance and 24-hour protein calculations undertaken at screening, baseline, Weeks 2 and 4, and monthly thereafter (or upon early termination). Bone marrow aspirations and/or tissue biopsy are also performed at Months 3, 6 and 12 if a patient's paraprotein serum concentration or 24-hour urine protein excretion declined to the next lower level, based on best response criteria. Preliminary results for the 28-day treatment period are summarized below.

Preliminary pharmacokinetic analyses based on these two studies indicated that AUC and C<sub>max</sub> values increase proportionally with dose following single and multiple doses in multiple myeloma patients (as was seen in healthy volunteers). Further, there was no evidence of accumulation with multiple dosing as single dose AUC<sub>(0-τ)</sub> was comparable to multiple dose AUC<sub>(0-τ)</sub> following the same dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Similar to healthy volunteer studies, double peaks were observed. Exposure in multiple myeloma patients appeared to be slightly higher based on C<sub>max</sub> and AUC values as compared to healthy male volunteers while clearance in multiple myeloma patients was lower than it was in healthy volunteers, consistent with their poorer renal function (both as a consequence of their age and their disease). Finally, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione half-life in patients was shorter than in healthy volunteers (mean 8 hours, ranging up to 17 hours).

In this study, the first cohort of 3 patients was treated for 28 days at 5 mg/day without any dose limiting toxicity (DLT). The second cohort of 3 patients subsequently commenced therapy at 10 mg/day. Patients in the second 10 mg/day of

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3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione cohort tolerated treatment well.

#### 6.5.3 Treatment of Solid Tumors

Study with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) was conducted in patients with varying types of solid tumors, including malignant melanoma (13), carcinoma of the pancreas (2), carcinoid-unknown primary (1), renal carcinoma (1), breast carcinoma (1) and NSCLC (2). Patients received 5 mg/day 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione for seven days and are subsequently escalated every seven days to 10 mg/day, 25 mg/day, and 50 mg/day for a total of 4 weeks of treatment. Patients who, experienced clinical benefit were permitted to continue on treatment as Named Patients.

The study initially enrolled 20 patients and was subsequently amended to enroll 16 additional patients (adrenal carcinoma, NSCLC, malignant mesothelioma, breast cancer, malignant melanoma (8), renal cell cancer (4)) at a higher dose. The 16 additional patients were given weekly escalating doses of 25 mg/day, 50 mg/day, 75 mg/day, 100 mg/day, 125 mg/day, and 150 mg/day over a 6-week period with continuing treatment for an additional six weeks.

The study of Phase 1 study was designed to determine a maximum tolerated dose (MTD) of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in patients with refractory solid tumors and/or lymphoma, as well as to characterize the pharmacokinetic and side effect profiles of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione in this patient population. The study design dictates that at least 3 patients must be enrolled at a dose level and have completed 28 days of treatment prior to enrollment of patients at the next higher dose level. Patients in the first cohort began dosing at 5 mg/day of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione. Patients will be escalated to 10, 20, 25, and 30 mg/day provided there is no toxicity.

In this study, the MTD is defined as the highest dose level in which fewer than two of six patients treated did not experience Grade 3 or greater non-hematological toxicity or Grade 4 or greater hematological toxicity. If, at any given dose level in either study, one out of three patients experiences toxicity, three additional patients must be treated at that particular dose. If, however, two out of six patients experience DLT, the MTD is judged to have been exceeded. No further dose escalations are to occur and additional patients are to be enrolled at the previous dose level. The dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione administered is escalated until the MTD is achieved or the maximum daily dose of is reached.

No DLTs were reported in the initial group of 20 patients enrolled in the study. Thirteen of the original 20 trial patients, along with 2 non-trial patients, continued on treatment as named patients at doses up to 150 mg/day.

#### 6.5.4 Treatment of Gliomas

This study was performed to find toxicity in patients with recurrent, high-grade gliomas. The study is designed such that patients are given increasingly higher doses of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione until a maximum tolerated dose (MTD) is established. The study also seeks to obtain preliminary toxicity information and pharmacokinetic data on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione, as well as to develop exploratory data concerning surrogate end points of angiogenic activity in vivo using functional neuro-imaging studies, and in vitro assays of serum angiogenic peptides.

Patients enrolled in the first cohort receive 2.5 mg/m<sup>2</sup>/day for a 4-week cycle. During each 4-week cycle of therapy, 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,

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6-dione administered once daily for 3 weeks followed by a week of rest. Patients who complete a treatment cycle may receive another cycle of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione treatment if two criteria are met. First, the patient must have stable disease or have experienced a partial response or complete response, or the patient is benefiting from the therapy with 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione as evidenced by a decrease in tumor-related symptoms such as neurological deficits. Second, the patient must have recovered from toxicity related to 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione which occurred in the prior cycle by Day 42 or sooner (28-day cycle plus limit of 2 weeks to recover) as evidenced by a return to Grade ≤1 toxicity level. Patients who experience DLT in the previous cycle should have their dose modified. DLT is defined as an non-hematological event Grade ≥3 toxicity or hematological event of Grade 4 toxicity thought to be related to the study medication. Patients who experience DLT in the first cycle and have no response to therapy are removed from the study.

3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione doses are subsequently escalated to 5, 8, 11, 15, and 20 mg/m<sup>2</sup>/day to a maximum total daily dose of 40 mg. Patients continue to receive 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione on a 4-week cycle per dose level until one of the off-study criteria are met.

Three patients are enrolled in each cohort. If at least one DLT occurs, three additional patients are added to the cohort at that particular dose level. If two DLTs occur, the MTD, defined as the dose at which fewer than one-third of patients at each dose level experiences DLT has been exceeded and four more patients are treated at the previous dose.

Patients who experience DLT during the first 4-week cycle are removed from the study, except if they have a response to therapy. For patients who have completed their first 4-week cycle of without DLT, but who subsequently experience Grade 3 or 4 hematological and/or nonhematological toxicity, treatment is suspended for a minimum of a week. If the toxicity resolves to <Grade 2 within three weeks, the patient is treated at two dose levels lower than the dose that caused the toxicity (or a 50% reduction if the patient was treated at the first or second dose level). Patients in whom Grade 3 or 4 toxicity does not resolve to <Grade 1 within three weeks, or those who have another Grade 3 toxicity at the reduced dose are removed from the study.

Pharmacokinetic sampling is performed prior the first dose of 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Day 1) and 0.5, 1, 2, 4, 6, 8, 24, and 48 hours thereafter. Sampling is also conducted pre-dose on Days 7 and 21 and 0.5, 1, 2, 4, 6, 8, and 24 post-dose on Day 21 to evaluate steady-state 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione levels.

#### 6.5.5 Treatment of Metastatic Melanoma

Patients with metastatic melanoma were started on 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione (Revimid™) at 5 mg/day for seven days. The dose was then increased every seven days to 10 mg/day, 25 mg/day, and 50 mg/day, respectively, for a total of four weeks on therapy. Five of the 13 melanoma patients who were treated under this regimen either showed disease stabilization or a partial response in the first four weeks of treatment. Tumor response was seen in cutaneous and subcutaneous lesions (five patients), lymph nodes (two patients), and liver (one patient). The duration of response was approximately six months. The result suggests that the compound appears is a promising new anti-cancer agent and has both antiangiogenic and immunomodulatory properties.



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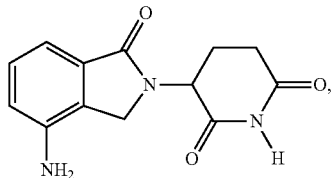
### 6.5.6 Treatment of Relapsed or Refractory Multiple Myeloma

Patients with relapsed and refractory Dune-Salmon stage III multiple myeloma, who have either failed at least three previous regimens or presented with poor performance status, neutropenia or thrombocytopenia, are treated with up to four cycles of combination of melphalan (50 mg intravenously), an immunomodulatory compound of the invention (about 1 to 150 mg orally daily), and dexamethasone (40 mg/day orally on days 1 to 4) every four to six weeks. Maintenance treatment consisting of daily an immunomodulatory compound of the invention and monthly dexamethasone are continued until the disease progression. The therapy using an immunomodulatory compound of the invention in combination with melphalan and dexamethasone is highly active and generally tolerated in heavily pretreated multiple myeloma patients whose prognosis is otherwise poor.

The embodiments of the invention described above are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials, and procedures. All such equivalents are considered to be within the scope of the invention and are encompassed by the appended claims.

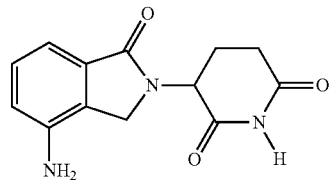
What is claimed is:

1. A method of treating multiple myeloma, which comprises administering in a 28 day cycle to a patient having multiple myeloma: (a) about 1 to about 50 mg per day of a compound having the formula:



or a pharmaceutically acceptable salt, solvate or stereoisomer thereof for 21 consecutive days followed by seven consecutive days of rest from administration of said compound, and (b) a therapeutically effective amount of dexamethasone during such 28 day cycle, wherein the patient has not received previous treatment for multiple myeloma.

2. The method of claim 1, wherein the compound is



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and is not a pharmaceutically acceptable salt, solvate or stereoisomer thereof.

3. The method of claim 1, wherein the compound is a pharmaceutically acceptable salt.

4. The method of claim 1, wherein the compound is a pharmaceutically acceptable solvate.

5. The method of claim 1, wherein the compound is a pharmaceutically acceptable stereoisomer.

6. The method of claim 1, wherein the patient is ineligible for stem cell transplantation.

7. The method of claim 1, wherein the patient is 65 years of age or older.

8. The method of claim 1, wherein the patient is younger than 65 years of age.

9. The method of claim 6, wherein the patient is younger than 65 years of age.

10. The method of claim 1, wherein the compound is administered in an amount of about 25 mg per day.

11. The method of claim 1, wherein the compound is administered in an amount of about 20 mg per day.

12. The method of claim 1, wherein the compound is administered in an amount of about 15 mg per day.

13. The method of claim 1, wherein the compound is administered in an amount of about 10 mg per day.

14. The method of claim 1, wherein the compound is administered in an amount of about 5 mg per day.

15. The method of claim 1, wherein the compound is administered in an amount of about 2.5 mg per day.

16. The method of claim 1, wherein the compound is administered orally.

17. The method of claim 16, wherein the compound is administered in the form of a capsule or tablet.

18. The method of claim 17, wherein the compound is administered in a capsule in an amount from about 1 mg to about 50 mg.

19. The method of claim 18, wherein the compound is administered in a capsule in an amount of about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg or about 25 mg.

20. The method of claim 17, wherein the capsule comprises the compound, lactose anhydrous, microcrystalline cellulose, croscarmellose sodium and magnesium stearate.

21. The method of claim 1, wherein the dexamethasone is administered in an amount of 40 mg per day on days 1-4 of each 28 day cycle.

22. The method of claim 1, wherein the dexamethasone is administered in an amount of 40 mg per day on days 1, 8, 15 and 22 of each 28 day cycle.

23. The method of claim 1, which further comprises administering a therapeutically effective amount of an additional active agent.

24. The method of claim 23, wherein the additional active agent is melphalan, doxorubicin, vincristine, prednisone, cyclophosphamide, biacin, a proteasome inhibitor, or a combination thereof.

25. The method of claim 1, wherein the solvate is hydrate.

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